TRANSYLVANIAN REVIEW OF SYSTEMATICAL AND ECOLOGICAL RESEARCH

15.2

The Wetlands Diversity

Editors

Angela Curtean-Bănăduc & Doru Bănăduc

Sibiu - Romania
2013

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Published based mainly on some of the scientific materials presented at the fourth "Aquatic Biodiversity International Conference" - Sibiu/Romania 2013

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IN MEMORIAM

Eugene Pleasants Odum
(1913-2002)

Eugene Pleasants Odum was an American biologist well known for his pioneering subtle approach, hard work and very valuable results on ecosystem ecology.

Born in September 1913, while his mother was in holiday on Lake Sunapee in New Hampshire to escape the summer heat of Athens, Georgia (USA), he was the son of Howard Washington Odum, a respected scholar in social justice, whose studies of integrated concepts, were certainly an influence on, his son's holistic approach and concepts.

Eugene Odum's studies in zoology started at the age of 15 at the University of North Carolina, when he developed a major interest in ornithology and avian ecology that was to last all his life. Eugene and Martha Huff Odum, whom he had met as a student, moved to the University of Georgia in 1940, where he served his entire career.

In 1946, trying to highlight the fact that ecology was not simply a basic discipline of biology, he began writing the first edition of Fundamentals of Ecology (1953). Later in his life he admited that one of his most satisfying accomplishments was his success in revealing that ecology is not a subdiscipline of biology but a stand-alone discipline.

He worked with his younger brother Howard Thomas Odum, on several major projects including: the second edition of Fundamentals of Ecology, published in 1959; The energetic Basis for Valuation of Ecosystem services, published in 2000. Through her skilled paintings, his wife enriched Eugene′s perspective of nature. Eugene and Martha′s son was a professor of environmental science at the University of Virginia.

Eugene Pleasants Odum carrer shows a clear uninterrupted intellectual growth, evolving from a bird ecologist to an ecosystem ecologist and overall a holistic thinker in his late years.

He supported the establishment of the Savannah River Ecology Laboratory, the Sapelo Island Marine Institute, and the world famous Institute of Ecology.

He stressed the role of mutualism, the mechanism of ecosystem development, and energetics as a comon denominator across different levels of organization.

He was also a philanthropist providing funding for his programmes, an environmentalist recognizing the need to protect ecological systems, and a teacher promoting integrative science.

He was a relentless birdwatcher, a tireless traveler, and an enthusiastic organic gardener until his death.

Although encouraged to retire officially in 1984, he continued to devote his time to writing, contributing to research publications and the fifth edition of Fundamentals of Ecology (2005).

In addition to his reputation as a research ecologist, *Eugene Pleasants Odum* also became a respected environmentalist during the decade of environment and was quoted frequently in Time, Newsweek amd Life magazines.

He played a major role in the passing of the Coastal Marshlands Protection Act of 1990 in the State of Georgia.

He designated in his will that more than half his 26 acre estate at Beech Creek be placed in permanent conservation protection, thus providing habitat for the wildlife he loved.

Eugene Pleasants Odum's legacy of generosity, subtle intelligence and capacity of understanding nature, will benefit generations in pursuit of education, research and service of nature.

The Editors

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BIOTOPES

BIOCOENOSIS

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Preface

In a global environment in which the climate changes are observed from few decades no more only through scientific studies but also through day by day life experiences of average people which feel and understand allready the presence of the medium and long-term significant change in the "average weather" all over the world, the most comon key words which reflect the general concern are: heating, desertification, rationalisation and surviwing.

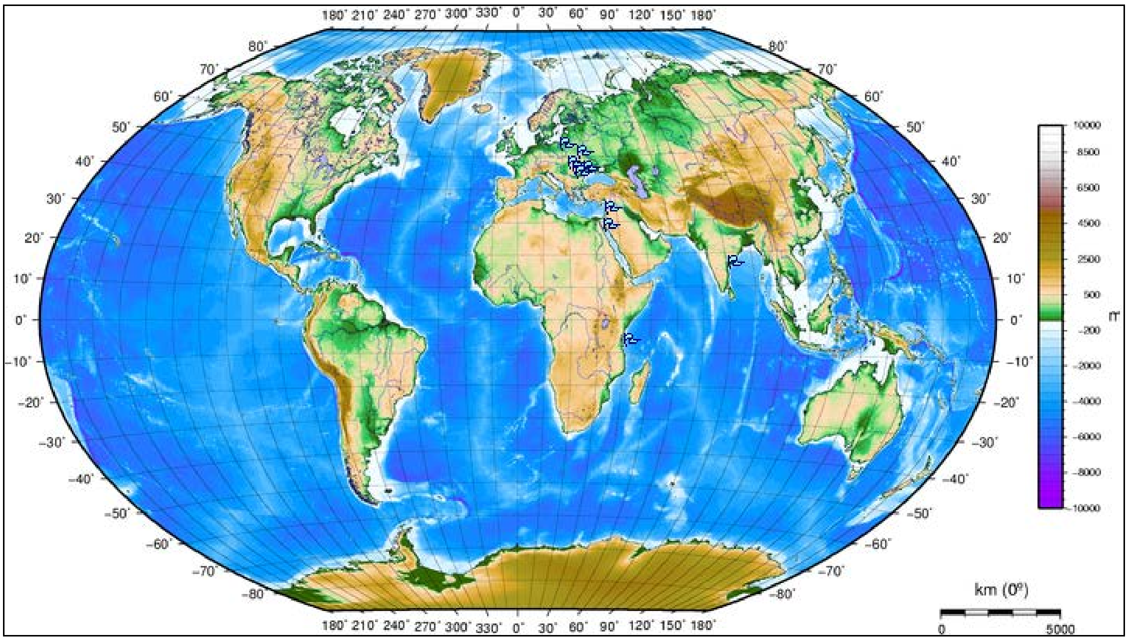
The causes, effects, trends and possibilities of human society to positively intervene to slow down this process or to adapt to it involve a huge variety of aproacess and efforts.

With the fact in mind that these aproaces and efforts shuld be based on genuine scientific understanding, the editors of the *Transylvanian Review of Systematical and Ecological Research* series launch a second annual volumes dedicated to the wetlands, volumes resulted mainly as a results of the *Aquatic Biodiversity International Conference,* Sibiu/Romania, 2007-2011.

The therm wetland is used here in the acceptance of the Convention on Wetlands, signed in Ramsar, in 1971, for the conservation and wise use of wetlands and their resources. **Marine/Coastal Wetlands -** Permanent shallow marine waters in most cases less than six metres deep at low tide, includes sea bays and straits; Marine subtidal aquatic beds, includes kelp beds, sea-grass beds, tropical marine meadows; Coral reefs; Rocky marine shores, includes rocky offshore islands, sea cliffs; Sand, shingle or pebble shores, includes sand bars, spits and sandy islets, includes dune systems and humid dune slacks; Estuarine waters, permanent water of estuaries and estuarine systems of deltas; Intertidal mud, sand or salt flats; Intertidal marshes, includes salt marshes, salt meadows, saltings, raised salt marshes, includes tidal brackish and freshwater marshes; Intertidal forested wetlands, includes mangrove swamps, nipah swamps and tidal freshwater swamp forests; Coastal brackish/saline lagoons, brackish to saline lagoons with at least one relatively narrow connection to the sea; Coastal freshwater lagoons, includes freshwater delta lagoons; Karst and other subterranean hydrological systems, marine/coastal. **Inland Wetlands -** Permanent inland deltas; Permanent rivers/streams/creeks, includes waterfalls; Seasonal/intermittent/irregular rivers/streams/creeks; Permanent freshwater lakes (over eight ha), includes large oxbow lakes; Seasonal/intermittent freshwater lakes (over eight ha), includes floodplain lakes; Permanent saline/brackish/alkaline lakes; Seasonal/intermittent saline/brackish/alkaline lakes and flats; Permanent saline/brackish/alkaline marshes/pools; Seasonal/intermittent saline/brackish/alkaline marshes/pools; Permanent freshwater marshes/pools, ponds (below eight ha), marshes and swamps on inorganic soils, with emergent vegetation water-logged for at least most of the growing season; Seasonal/intermittent freshwater marshes/pools on inorganic soils, includes sloughs, potholes, seasonally flooded meadows, sedge marshes; Non-forested peatlands, includes shrub or open bogs, swamps, fens; Alpine wetlands, includes alpine meadows, temporary waters from snowmelt; Tundra wetlands, includes tundra pools, temporary waters from snowmelt; Shrub-dominated wetlands, shrub swamps, shrub-dominated freshwater marshes, shrub carr, alder thicket on inorganic soils; Freshwater, tree-dominated wetlands; includes freshwater swamp forests, seasonally flooded forests, wooded swamps on inorganic soils; Forested peatlands; peatswamp forests; Freshwater springs, oases; Geothermal wetlands; Karst and other subterranean hydrological systems, inland. **Human-made wetlands -** Aquaculture (e. g., fish/shrimp) ponds; Ponds; includes farm ponds, stock ponds, small tanks; (generally below eight ha); Irrigated land, includes irrigation channels and rice fields; Seasonally flooded agricultural land (including intensively managed or grazed wet meadow or pasture); Salt exploitation sites, salt pans, salines, etc.; Water storage areas, reservoirs/barrages/dams/impoundments (generally over eight ha); Excavations; gravel/brick/clay pits; borrow pits, mining pools; Wastewater treatment areas, sewage farms, settling ponds, oxidation basins, etc.; Canals and drainage channels, ditches; Karst and other subterranean hydrological systems, human-made.

The editors of the *Transylvanian Review of Systematical and Ecological Research* started and continue this new annual sub-series (*Wetlands Diversity*) as an international scientific debate platform for the wetlands conservation, and not to take in the last moment, some last heavenly "images" of a perishing world …

This sixth volume included variated researches from diverse wetlands around the world.



The subject areas (⚑) for the published studies in this volume.

No doubt that this new data will develop knowledge and understanding of the ecological status of the wetlands and will continue to evolve.

Acknowledgements

The editors would like to express their sincere gratitude to the authors and the scientific reviewers whose work made the appearance of this volume possible.

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(ISSN-L 1841 – 7051; online ISSN 2344 – 3219)

DE GRUYTER OPEN

ESTIMATING PARTICLE CONCENTRATION IN NATURAL WATER BY FAR FIELD SPECKLE INTENSITY

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DOI: 10.2478/trser-2013-0017

KEYWORDS: Coherent light-scattering, aqueous suspension, particle concentration, speckle intensity.

ABSTRACT

A coherent light-scattering experiment on an aqueous clay suspension having a concentration that stretches over an extended range was carried on. The far field speckle was recorded in an unconventional manner.

A computer code for image processing, written for this purpose, was used to extract the scattered light intensity. The variation of the average scattered light intensity with the particle concentration was analyzed, and a possible fast procedure for assessing the particle concentration is suggested.

ZUSAMMENFASSUNG: Abschätzung der Partikelkonzentration in natürlichem Wasser durch Messung der Intensität des kohärenten zerstreuten Lichtes.

Die Arbeit stellt ein kohärentes Lichtstreuungsexperiment vor, das in einer wässerigen Schlammflüssigkeit, mit einer Konzentration, die sich über einen großen Aktionsradius erstreckt, durchgeführt wurde. Das Interferenzbild wurde auf unkonventionelle Art und Weise als Film aufgezeichnet.

Um die Streulichtintensität zu berechnen, wurde ein spezieller, eigens für dieses Experiment geschriebener PC Kode für Bildbearbeitung benutzt. Die Variation der Streulichtintensität in Abhängigkeit von der Partikelkonzentration wurde beobachtet und als Ergebnis ein rasches Verfahren zur Schätzung der Partikelkonzentration vorgeschlagen.

REZUMAT: Estimarea concentrației de particule în apa naturală prin măsurarea intensității luminii coerente împrăştiate.

A fost efectuat un experiment de împrăştiere a luminii pe suspensie de nămol având o concentraţie care se întinde pe mai multe ordine de mărime. Imaginea de interferenţă a fost înregistrată într-o manieră neconvenţională.

Intensitatea medie a luminii împrăştiate a fost extrasă din înregistrare folosind un program scris în acest scop. A fost analizată variaţia intensităţii medii cu concentraţia de particule. Ca rezultat este sugerat un procedeu rapid de estimare a concentraţiei.

INTRODUCTION

Natural water contains particles in suspension, which causes water opacity. This physical property of natural water is named turbidity (***, 2002). Suspended particles can be clay, sand, silt, algae, plankton, micro-organisms and other substances (***, 2006). Suspended particles absorb visible light and this causes the increase of the particle temperature. Heat is transferred to water; therefore temperature increases faster in turbid water than it does in clear water under the same incident light intensity. Gumpinger et al. (2010) stated that “water temperature is considered one of the most essential regulating parameters in aquatic ecosystems”. Moreover, because of the intensive interrelations with other physical and chemical parameters, water temperature has a high indicative value when considering the general condition of a river ecosystem (Gumpinger et al., 2010).

Turbidity is often used as an indicator of the total amount of material suspended in water, but is neither a measure of the concentration nor of the size of the particles. Nevertheless, good knowledge of the size and the type of the suspended particles is important, because the fine particles suspended in water can carry bacteria, excess nutrients and toxic materials, which might be a hazard for water.

Optical procedures for assessing the amount of particles in suspension are appealing because they are fast to perform and do not require physical or chemical sample processing. When coherent light crosses a medium having scattering centers (SC), a non uniformly illuminated image is obtained, currently named speckled image, as explained in a report of previous work on this subject (Chicea, 2013 a, b). The image is not static but changes in time giving the aspect of “boiling speckles” (Goodman, 1984; Briers, 2001).

The speckled image can be observed either in free space, in which case it is named objective speckle (Goodman, 1984) or far field speckle (Briers, 2001), or on the image plane of a diffuse object, in which case it is named subjective speckle (Goodman, 1984) or image speckle (Briers, 2001). In this work, the objective speckle, respectively far field speckle is considered as in Chicea (2013 a, b).

The dynamics of the speckle field was analyzed by correlometric methods (Boas and Yodh, 1997; Aizu and Asakura, 1991; Fedosov and Tuchin, 2001) or by laser speckle contrast analysis (Briers et al., 1999; Zimnyakov et al., 2002). The speckle size can be used to measure the roughness of a surface (Lehmann, 1999; da Costa and Ferrari, 1997; Berlasso et al., 2000) or to assess the thickness of a semi-transparent thin slab (Sadhwani et al., 1996). Giglio et al. (2001) and other papers report on the near-field speckle dependence of the particles size. The work reported by Piederrière et al. (2004 a, b) and Chicea (2010) used a transmission optical set-up to measure the far field. This type of experimental setup is used in the work reported here, which is a continuation of the previous experimental work (Chicea, 2013 a, b) but which uses a different procedure for data processing and for interpreting the results.

MATERIAL AND METHODS

The clay collected from the bottom riverbed of the Trinkbach River, that crosses Sibiu city, was diluted in water, allowed to sediment for 24 hours and the water was discarded to remove the organic suspension. The sediment was dried, weighed and then was mixed with deionised water to produce samples of clay particle suspension with different concentrations.

Prior to starting the light-scattering image experiment, a diluted suspension was subject to a Dynamic Light-scattering experiment using a modified procedure extensively described in previous papers (Chicea, 2010; Chicea, 2012 a; Chicea et al., 2012). The average diameter of the clay particles was found to be 0.6 μm, as previously reported (Chicea, 2013 b). The samples and the recordings are the same as in the previous work, but the data processing procedure is different, as presented further on. The scheme of the experiment and the detailed description of the experimental setup is presented in a previous article (Chicea, 2013 b), in figure 1 together with a frame of a recording, in figure 2 of the same article (Chicea, 2013 b); therefore they are not repeated here.

The average speckle size is calculated as the normalized autocovariance function of the intensity speckle pattern recorded in the observation plane (Piederrière et al., 2004 a, b). The average speckle contrast was analyzed in connection with the particle concentration and the results have already been presented (Chicea, 2013 b). In this paper an alternative approach is proposed to relate the particle concentration to the average scattered light intensity.

Unlike the work reported by Chicea (2012 b), where a punctual detector was used, a charged coupled device was used in this work and a video was recorded rather than a time series. A computer program was written to read the video recorded in a conventional format and to extract the level of the scattered light intensity. The program reads the video and extracts frame by frame in a 640 x 480 array of 24 bits intensity levels. We can note I(i,j) = I(xi, yj) the intensity recorded by the cell (i,j) of the CCD, hence by the pixel (i,j) of the array of pixels that make up the image. Once the array of intensities is extracted for each frame, the levels of intensity are averaged for that frame. The collection of intensity values is averaged for the whole recording and the average value I is considered to be the intensity value for that particular video, hence for that particular sample. The standard deviation for the collection of intensity values computed for one sample is considered to be the relative error in assessing the intensity for that particular sample; therefore the error bars in figure number 1 are the standard deviations.

A number of 22 samples having clay suspension with different concentrations were prepared. For each sample the cuvette was filled with the suspension and an MPEG type of video lasting for 30 seconds and containing 300 frames was recorded and processed as described above. The video was converted to an AVI format later on and processed as described above.

A simple physical procedure was also used to measure the actual amount of sediment in a sample, as an alternative and control method, although tremendously more time consuming. It consists of weighing an empty and freshly cleaned Petri dish, adding 50 ml of suspension in the dish and allowing it to slowly evaporate at a temperature around 60^oC that was controlled by adjusting the distance from the dish to a pair of 100 W light bulbs. In the work previously reported (Chicea, 2013 b), there was only one light bulb that was used as a heat source while for this work we used a pair of bulbs to increase the temperature. Moreover, the temperature was risen to 60^oC and this decreased the time to dry the sample. We did not pass 60^oC as there is the risk to produce fast evaporation during the temperature adjustment procedure and this might evacuate the very small particles and produce a systematic error in weighing the amount of dry clay. After the water evaporation was completed, the dish with the sediment was weighed again and the difference represented the sediment mass. More details on this method and on assessing the relative error of the method are presented in the previous paper on this subject (Chicea, 2013 b).

RESULTS AND DISCUSSION

The samples were prepared using the procedure described in the previous section starting with a concentration of 7.5 g/l and decreasing it by successive dilution down to 0.081 g/l. This concentration range is quite extended, as it covers two orders of magnitude. The average scattered light intensity was computed for each sample and the average scattered light intensity versus clay concentration is presented in figure number 1.

We notice that in figure number 1 the average intensity, I, exhibits an increasing trend in the very small concentration range, up to 0.35 g/l. From 0.35 g/l up to 6.67 g/l the average intensity decreases monotonously. As the concentration increases above 6.67 g/l the average intensity exhibits an increasing trend again. Overall, the curve that can be plot through the data points is smooth and the error bars are small relative to the range of average intensity values.

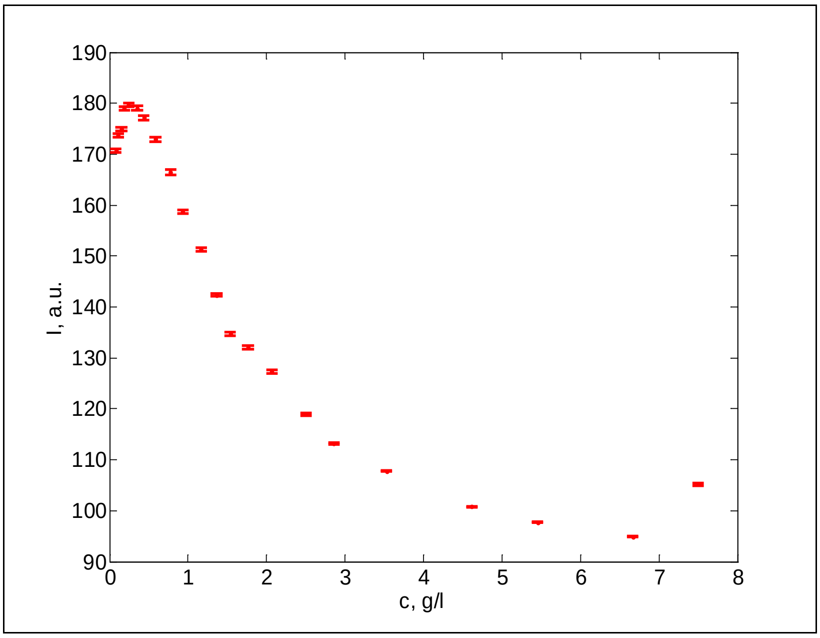

Figure 1: The average scattered light intensity versus clay concentration (c); intensity is expressed in arbitrary units (a.u.) and concentration in grams per litre (g/l).

This result is similar to the results reported by Piederrière et al. (2004 a) on latex microspheres, although at a first look Piederrière et al. (2004 a) presents an almost linear decrease. This can be explained by the much bigger concentration range used in this work. The work presented in Piederrière et al. (2004 a) represents just a small area of the extended decreasing part of the curve in figure number 1. Examining the plot of average intensity by concentration we notice that the monotone interval can be used as a calibration curve for measuring the concentration in the 0.35-6.67 g/l concentration range.

The procedure was tested on four samples obtained by mixing two unmeasured volumes of different diluted samples, therefore the clay concentration being unknown, following the procedure presented in Chicea (2013 b). The samples were analyzed both by using the curve in figure number 1 as a calibration and by evaporation followed by weigh difference. The results are presented in table number 1, where the first column is the clay concentration measured by the sediment mass, after evaporation, divided by the 50 ml volume; the second column contains the concentration measured by average intensity assessment; the third column contains the error of one method relative to the other, considering the weigh method as reference; and the fourth column contains the error of the weight difference method.

The slope of the curve in figure number 1 in the monotone range is bigger in the smaller clay concentration range, which makes the average intensity method more precise in this range, where the weight difference method has a lower precision, as explained in detail in a previous paper (Chicea, 2012 b) and proved by the fourth column in table number 1.

The procedure described in the material and method section resembles to a certain degree the physical procedure previously described (Chicea, 2012 b), yet it is essentially different. In this work image recording using a webcam and a computer followed by image processing is used, while the work previously reported was carried on using a detector, a data acquisition system, a computer, and it required time series processing.

Table 1: The concentration of four unknown samples and the errors in measuring the concentrations.

Concentration g/l (weight difference)	Concentration g/l (< I >)	Error between methods (%)	Error in weight difference (%)
0.38	0.39	2.56	10.5
0.76	0.79	3.8	5.2
2.5	2.36	5.93	1.6
5.1	5.6	8.93	0.8

CONCLUSIONS

A simple physical procedure using the far interference field of a coherent light beam incident on a suspension can be used to assess the clay concentration in natural water over a concentration range that covers more than one order of magnitude. The average scattered light intensity is extracted and computed using a program written for this purpose. The average intensity variation with the clay concentration was found to be monotone over an extended concentration range, specifically from 0.35 g/l to 6.67 g/l. This is a range with a relatively big concentration in natural water, as the water sample is opaque even for a 12 mm thick cuvette. For such a big concentration turbidity is not measurable with the simple method using the Secchi disk (***, 2002). The monotone part of the plot in figure number 1 can be used as a calibration curve. An unknown sample can be used as a target for the laser beam and a video can be recorded, the average intensity can be measured using the above mentioned procedure and the monotone part of the plot in figure number 1 can be used to assess the clay concentration in that water sample.

The method proposed in this article is very fast, as it does not require chemical or physical sample processing, but simply putting the sample in a cuvette, recording and processing the video. Once the average scattered intensity is computed, finding the concentration from the plot in figure number 1 is straightforward. A refinement might consist in fitting a polynomial on the monotone part of the plot in figure number 1, considering the concentration versus intensity and using the polynomial for a direct calculation of the concentration from the computed average intensity. The above mentioned steps take less than ten minutes, once the calibration curve is ready to be used. Caution should be taken and a preliminary concentration assessment by sedimentation, evaporation and weighing the sediment should be performed, in order to make sure that the concentration is in the monotone range of figure number 1. A faster, but empirical procedure to make sure of this is to simply look at the 12 mm thick cuvette filled with the sample; this should not look completely transparent, like deionised water, because in this case the concentration is below the starting part of the monotone portion of the plot, that is below 0.35 g/l.

ACKNOWLEDGMENTS

Many thanks to the environmental sciences group of the Faculty of Sciences at the “Lucian Blaga” University of Sibiu for fruitful discussions and for clarifying certain issues related to physical factors involved in environment protection. I am also deeply indebted to the *Transylvanian Review of Systematical and Ecological Research* editors, Curtean-Bănăduc A. and Bănăduc D., for their continuous support and encouragement to carry on experimental work related to environment assessment and monitoring.

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GEOGRAPHICAL AND HUMAN IMPACT ELEMENTS INFLUENCE ON THE FISH FAUNA OF THE OLTEŢ RIVER (ROMANIA)

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DOI: 10.2478/trser-2013-0018

KEYWORDS: lotic biotopes, fish associations, Carpathian fish integrity index.

ABSTRACT

A white spot regarding the fish fauna of the upper and middle Olteț River was covered and the significant changes appeared in this respect and in the lower course were identified. In general the passing of the river from one type of geographical/relief type unit to another, induce the changing of the ichthyological zone and the increasing of the fish species number. There where this natural situation did not appear is because different human impact causes create unnatural variations in fish communities’ compositions and individuals’ number. The presence of a various human impact especially downstream the mountain area makes to exist here a relative unbalanced fish fauna structure, in comparison with the mountainous sector. Overall, the geographical and human impact elements, and a result of them, the self cleaning capacity of river, are main driving forces which induce the fish fauna composition variability.

ZUSAMMENFASSUNG: Der Einfluss geographischer Faktoren und menschlicher bedingter Faktoren auf die Fischfauna des Olteţ-Flusses (Rumänien).

Mit vorliegender Arbeit wurde ein weißer Fleck bezüglich der Fischfauna des oberen und mittleren Olteţ-Flusses ausgefüllt und dabei auch die dadurch aufgetretenen, signifikanten Veränderungen am Unterlauf identifiziert. Im allgemeinen führt der Übergang des Flusses von einer geographischen Einheit bzw. von einem Relieftyp zu einem anderen zu einem Wechsel der ichtyologischen Zone und einem Anwachsen der Anzahl von Fischarten. Warum diese natürliche Situation nicht auftritt, ergbit sich aus unterschiedlichen, menschlich bedingten Eingriffen, die unnatürliche Variationen in der Zusammensetzung der Fischgemeinschaften und Individuenzahl verursachen. Das Vorhandensein unterschiedlicher, menschlich bedingter Veränderungen, vor allem unterhalb des Gebirgsabschnittes führt dazu, dass hier im Vergleich zu den oberen Abschnitten des Flusses eine relativ unausgewogene Fischfaunastruktur vorzufinden ist. Überall sind die geographischen und menschlich bedingten Elemente und das Ergebnis ihrer Auswirkungen auf die Selbstreinigungskapazität des Flusses die Hauptkräfte, die Variabilität der Zusammensetzung der Fischfauna bestimmen.

REZUMAT: Influența elementelor geografice și antropice de impact asupra ihtiofaunei din râul Olteț (România).

A fost studiată ihtiofauna din cursul superior și mijlociu al râului Olteț, o zonă necercetată din acest punct de vedere până în prezent, de asemenea au fost evaluate schimbările în structura comunităților de pești față de situația de acum 50 de ani (Bănărescu, 1964) în cazul cursului inferior, evidențiindu-se modificări semnificative. În general, trecerea unui curs de apă de la un tip de unitate geografică sau de relief la alta face ca diversitatea ihtiocenozelor să crească spre aval. În zonele afectate de diverse tipuri de impact antropic, această tendință naturală nu se manifestă, iar structura comunităților de pești prezintă deviații majore de la starea naturală (în ceea ce privește compoziția specifică și numărul de indivizi). Prezența unei palete largi de tipuri de impact antropic, în special, în aval de zona montană duce la o structură a ihtiocenozelor relativ dezechilibrată prin comparație cu sectorul montan. Ca tendință generală, factorii de influență geografici și antropici și rezultanta lor, capacitatea de autoepurare a râului sunt principalele forțe care condiționează variabilitatea structurii comunităților de pești.

INTRODUCTION

Grigore Antipa (1867-1944) the first great Romanian ichthyologist, realized the fact that Romania should have a nationwide territory approach in the fish fauna study. In order to help manage this very important natural resource, the first publication regarding the fish fauna of Romania was created (Antipa, 1909).

Petru Mihai Bănărescu (1921-2009), the greatest Romanian ichthyologist, deepened and extended this national approach, realizing the astonishingly publication regarding the fish fauna of Romania (Bănărescu, 1964), with an impressive quantity and quality of data, many of these data actual even today, after a half of a century.

Many researchers have worked, in this field of study, and have tried to complete this work, but the task is far from over due to relatively big Romanian national territory and mostly due to its high geographical variability. This variability induces a relatively high variability of the aquatic biotopes and of their ichthyocenosis, and due to the continuous and various human impacts on fish fauna habitats, the fish fauna variability also increases.

In this general context, it is easy to accept the need and the actuality of such inventory/assessment ichthyological studies, especially in rivers where: no data are available, rivers where long/significant sectors have no data available, or where the human impact and/or climate changes induced modifications in the fish habitats. Thus, new qualitative and quantitative fish fauna related aspects have appeared. Essentially, the fish communities' assessment, monitoring, management, protection, conservation, etc., cannot be done properly without such complete ichthyological surveys, as they are an initial data base for future comparisons. The numerous protected areas proposed and "managed" on fake data regarding their fish fauna is just an example of some of the unfortunate assessment, monitoring, management, protection and conservation of fish "on paper".

The Olteţ River fish fauna fits this type of situation, it was studied by Bănărescu (1964) in its very low sector; a sector which suffered intensive human impact, especially in the last half of the XXth century. Upstream, this relatively short studied lower sector (Balș locality - Olteț-Olt rivers confluence), a sector negatively affected by pollution, hidrotechnical works and habitats change. Until now, no ichthyologist has studied the river in the rest of its significant length. In spite of this situation, for example, and based on "theoretical" fish data, the "Cheile Oltețului"/"Oltețului Gorge" Protected Natural Area was proposed and designated to conserve fish species which never lived there.

This intensive (two years long sampling campaign) and extensive (the distance between the sampling stations are between one to three and a half km) ichthyological study, serves as the first data base, for this important Carpathian river.

The Olteţ River is the main tributary of the 615 km long Olt River, one of the biggest affluent of the lower Danube River. The Olt Watershed (24.439 km^2; 10.1% of the Romanian national territory) is situated in the central and southern part of Romania; springing in the Oriental Romanian Carpathians, passing to the west the Transylvanian Depression, south of the Meridional Romanian Carpathians, in the southern Romanian subcarpathians and the Romanian Plain until the convergence with the Danube (Diaconu and Stănculescu, 1971; Roşu, 1980; Posea, 1982; Badea, 1983; Sommerwerk et al., 2009).

The upper and middle Olt Basin fish fauna was studied recently (Bănăduc, 1999, 2000, 2001; Bănăduc and Curtean-Bănăduc, 2002; Curtean-Bănăduc and Bănăduc 2004; Curtean-Bănăduc et al., 2014), the situation being significantly different in the lower part of the basin.

The Olteţ River springs in the Căpăţânii Mountains, which belong to the Parâng Massif and to the Romanian Meridional Carpathians; the mountainous area is limited at the east by the Olt River and in the west by the Jiu River (Velcea and Savu, 1982). It has a length of 615 km and a basin surface of 24,000 km^2 (Posea et al., 1982).

The Olteţ River basin is situated in the south-southwest part of Romania (Fig. 1), flowing from north to south in its upper sector and from north-west to south-east in its middle and especially in its lower part, passing the administrative units Gorj, Vâlcea and Olt.

The Olteţ Valley geology is a varied one (different crystalline rocks, old and new eruptive rocks, sedimentary rocks, etc.) (Velcea and Savu, 1982), and is reason for which this river has a relatively high natural relief units variability (Fig. 2) and riverbed biotopes/habitats variability from its springs to the confluence with the Olt River.

The Olteţ Basin shelter also some of the oldest (Quaternary) prehistoric man tools in the Romanian territory (Pebble-Culture) (Mutihac, 1990), the continuous presence of man in this basin being reflected mostly due to the later human developments.

The **mountainous sector** (❶) with crystalline schists, volcanic and calcareous rocks, is characterized by a typical mountainous lotic habitats with steep slopes, deepened and accentuated "V" and "U" profiles of the valley, big stones in the riverbed, waterfalls, and very fast flowing water. The Jurassic calcareous massif in the northern proximity of Polovragi locality, allows for the possibility of this river to cut one of the most spectacular gorges in the Carpathians, the "Cheile Olteţului"/"Olteţului Gorge" (Ujvári, 1972).

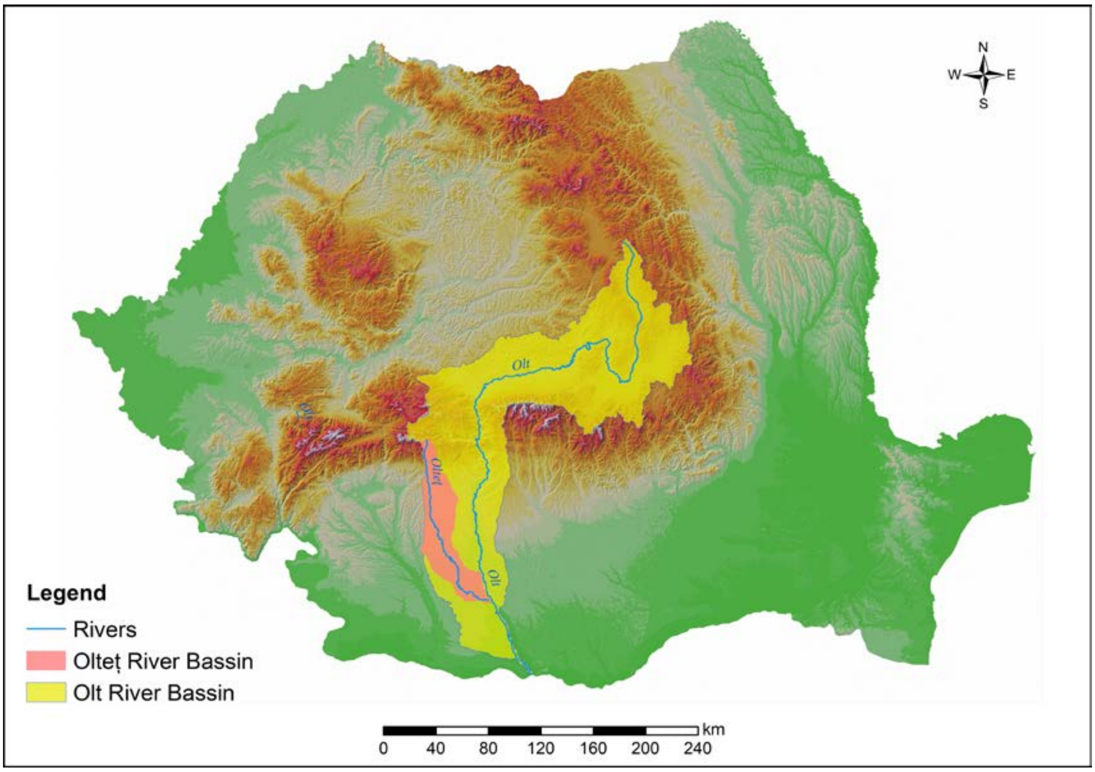

Figure 1: Localization of the Olteţ and Olt basins, in the Romanian national territory.

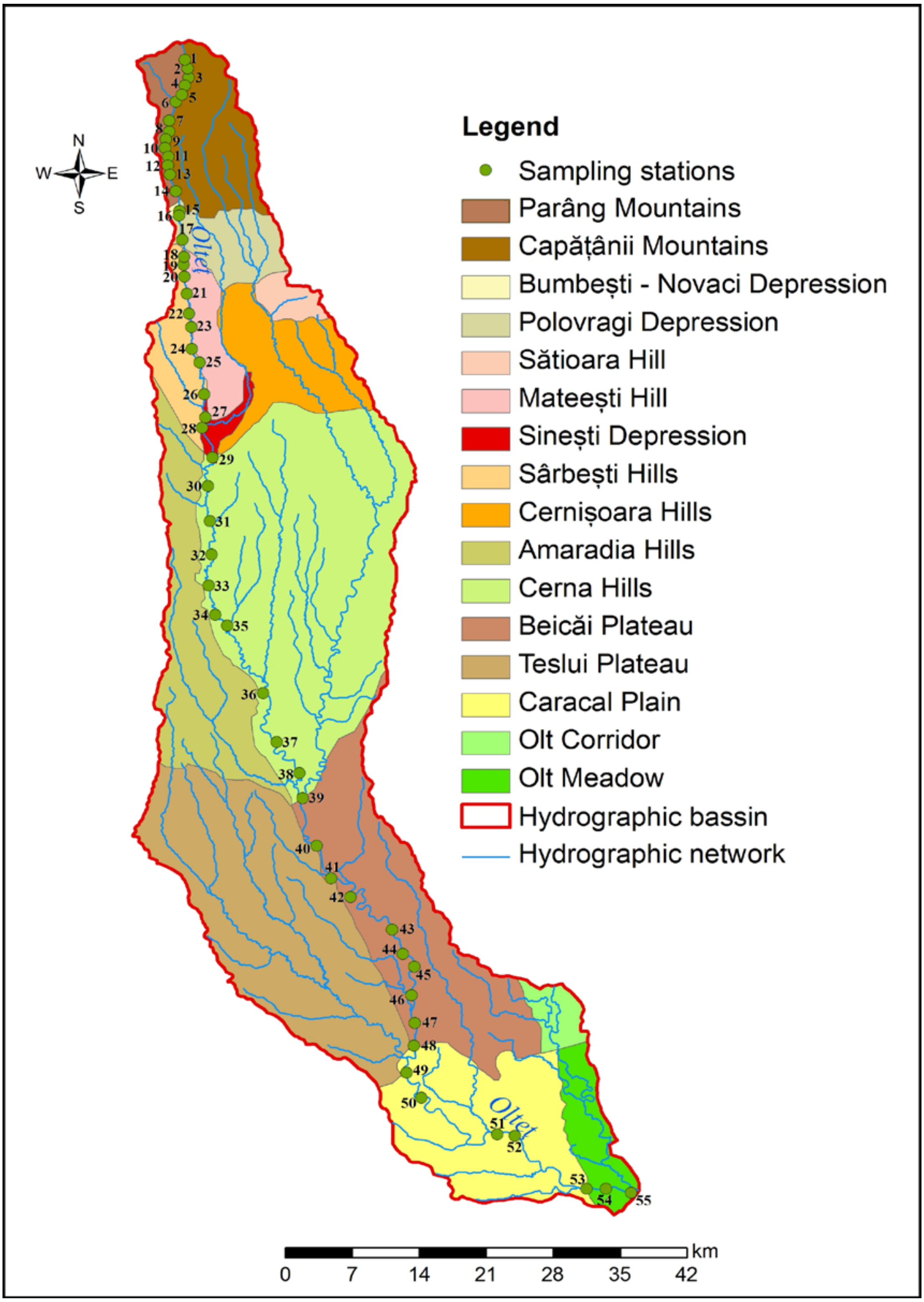

Figure 2: Relief units and sampling ststions
in the the Olteţ River basin area.

Downstream the “Cheile Olteţului”/“Olteţului Gorge”, the river passes the **subcarpathian Polovragi Depression/Depresiunea Polovragi sector** (❷), with Meotian clay in the geological substrate (Tufescu, 1966), and boulders and stones in the riverbed due to the continuous erosion and transport of these materials from the upper near mountain sector of the valley. The “V/U” shaped valley still allows the water to flow fast.

Downstream the subcarpathian Polovragi Depression the valley passes on to the **Getic Depression/Depresiunea Getică sector** (❸); the river eroded a big gravel geological substratum known as “Pietrişuri de Cândeşti”/“Cândeşti Gravels” and deposited there in a lotic-lenitic regime (Roşu, 1973). The river is in a submountainous (fast velocity) regime.

Downstream, the river passes through the unit named **Getic Piedmont/Piemontul Getic sector** (❹). Characterized by gravels, sands and in some sectors, conglomerates, (Tufescu, 1966), the water flow speed becomes moderate.

The final sector of the Olteţ River, before its confluence with the Olt River and, in the proximity of Fălcoiu Commune, is a plain relief belonging to the **Romanian Plain/Câmpia Română sector** (❺). Characterized by Pliocen and Cuaternary, old sedimentary sands, gravels, clay and loess, the water flow speed becomes low, even stagnant in some areas near banks.

MATERIALS AND METHODS

This ichthyological study was realized on the whole 175 km length of the Olteţ River, from its mountainous area to the confluence with the Olt River, in 2011-2012.

The distances between two consecutive sampling stations where around between one to three and a half km, 56 sampling stations (55 quantitative and one qualitative) were done.

The fishing method which was used was the electrofishing in time/effort unit (30 minutes). The device which was used was an Aquatech IG 600, 30 A, 0.65/1.2 kw, with two net stopers. All the sampled fish were identified, counted and released immediately in situ.

For the fish associations, localization was used a “Garmin GPS map 62s”.

The Carpathian Fish Index of Biotic Integrity (CF-IBI) (Bănăduc and Curtean-Bănăduc, 2002) was used in discriminating human induced degradation effects on ichthyofauna integrity, based on life history and feeding relationships selected metrics.

RESULTS

❶ The most upper Olteţ River area, in the **mountainous sector**, from its springs to the exit of the “Olteţului Gorge”/“Cheile Olteţului” belongs to the upper trout zone (O_1-O_{13}). Here only *Salmo trutta fario* (Tab. 1) individuals were sampled, the explanation being based on the habitat characteristics like rapids, waterfalls, stony river bed, “V” shaped slopes, etc., belonging to two mountainous relief units namely Parâng and Căpăţânii mountains (Figs. 2, 3 and 4). Despite the valley being very narrow, there are few small lateral natural water accumulations (Fig. 5), with their permanent springs or formed by small tributaries in which fish associations of *Phoxinus phoxinus* and *Squalius cephalus* are present. The CF-IBI 45 score on the river reflects exceptional assemblage of fish species, comparable to pristine areas. The individual trout numbers and the presence of all age classes in the sampling sections reveal an excellent state of the river. The lower number of individuals in the station O_{13} can be explained by the fact that sometimes at low water levels, the riverbed dries out completely and the water sometimes will flow only underground through a well developed carstic substrata. The very difficult accessibility to the river due to high and steep slopes results in very low human impact which therefore induces an excellent state fish fauna.

Figure 3: Mountainous rocky stream sector characteristic habitat.

Figure 4: Olteţ Valley steep "V/U" shape slopes in the Cheile Olteţului/Olteţului Gorge.

Table 1: The upper trout zone area of Olteţ River (O_1-O_7); *Salmo trutta fario - S.t.f.*

Sampling stations	Sampling station localization and description	Species	Number of individuals	Abundance (%)
O_1	N - 45.3314 E - 23.7903 5 km upstream the confluence with Ungurelul tributary. Riverbed length maximum 3.1 m and minimum 2.1 m. Maximum water depth 13 cm.	*S.t.f.*	30	100
O_2	N - 45.3230 E - 23.7939 4 km upstream the confluence with Ungurelul tributary. Riverbed length maximum 3.3 m and minimum 2.2 m. Maximum water depth of 14 cm.	*S.t.f.*	42	100
O_3	N - 45.3061 E - 23.7907 3 km upstream the confluence with Ungurelul tributary. Riverbed length maximum 3.3 m and minimum 3.1 m. Maximum water depth of 21 cm.	*S.t.f.*	73	100
O_4	N - 45.3139 E - 23.7907 2 km upstream the confluence with Ungurelul tributary. Riverbed length maximum 4.1 m and minimum 2.9 m. Maximum water depth of 25 cm.	*S.t.f.*	68	100
O_5	N - 45.2971 E - 23.7868 1 km upstream the confluence with Ungurelul tributary. Riverbed length maximum 4.9 m and minimum 3.1 m. Maximum water depth of 32 cm.	*S.t.f.*	99	100
O_6	N - 45.2901 E - 23.7788 Downstrean the confluence of Olteţ and Ungurelul rivers. Riverbed length maximum 5.2 m and minimum 3.6 m. Maximum water depth 33 cm.	*S.t.f.*	33	100
O_7	N - 45.2717 E - 23.7705 1 km downstrean the confluence of Olteţ and Ungurelul rivers. Riverbed length maximum 8.4 m and minimum 4.9 m. Maximum water depth 39 cm.	*S.t.f.*	82	100

Table 1 (continuing): The upper trout zone area of Olteţ River (O_8-O_{13}); *Salmo trutta fario - S.t.f.*

Sampling stations	Sampling station localization and description	Species	Number of individuals	Abundance (%)
O_8	N - 45.2609 E - 23.7707 2 km downstrean the confluence of Olteţ and Ungurelul rivers. Riverbed length maximum 9.3 m and minimum 7.6 m. Maximum water depth 45 cm.	*S.t.f.*	60	100
O_9	N - 45.2536 E - 23.7667 3 km downstrean the confluence of Olteţ and Ungurelul rivers. Riverbed length maximum 9.9 m and minimum 7.8 m. Maximum water depth 43 cm.	*S.t.f.*	71	100
O_{10}	N - 45.2449 E - 23.7654 4 km downstrean the confluence of Olteţ and Ungurelul rivers. Riverbed length maximum 9.5 m and minimum 7.4 m. Water maximum depth 44 cm.	*S.t.f.*	51	100
O_{11}	N - 45.2364 E - 23.7702 5 km downstrean the confluence of Olteţ and Ungurelul rivers. Riverbed length maximum 7.3 m and minimum 5.1 m. Maximum water depth 60 cm.	*S.t.f.*	59	100
O_{12}	N - 45.2280 E - 23.7698 6 km downstrean the confluence of Olteţ and Ungurelul rivers. Riverbed length maximum 1.2 m and minimum 8.5 m. Maximum water depth 24 cm.	*S.t.f.*	61	100
O_{13}	N - 45.2190 E - 23.7725 7 km downstrean the confluence of Olteţ and Ungurelul rivers. Riverbed length maximum 8 m and minimum 4.2 m. Maximum water depth 80 cm.	*S.t.f.*	15	100

Figure 5: Small natural lateral water accumulation in the Olteţ upper basin.

❷ Downstream the “Olteţului Gorge”, where the river passes in the **subcarpathian Polovragi Depression sector** (O_{14}-O_{16}), is the lower trout zone, and *Salmo trutta fario* remains dominant with a decreasing trend to the lower sectors, but still influence the downstream zone due to the excellent state of the ichthyofauna in the mountainous area.

The upper part (O_{14}-O_{15}) is in a natural mountain-submontane transition condition (CF-IBI 45) regarding the present habitats (Figs. 6 and 7), reflected in a high number of trouts (Tab. 2). In O_{16} sector near Polovragi Commune, due to a chaotic exploitation of the riverbed minerals (Fig. 8), the CF-IBI score is 2 and reflects extreme few species and individuals present; tolerant species being dominant. The *Salmo trutta fario* disappeared and only few *Orthrias barbatulus* individuals were sampled in small lateral artificial cavities in the left riverbed/banks area (Fig. 9) that remained after the riverbed exploitation activities. The persistence of this species here is problematic due to aggressive human impact.

A large dam (Fig. 10) is near Polovragi Commune, in Polovragi Depression (O_{17}). A dam which deflects in a subterranean large pipe where the majority of the water flows for a series of two medium size downstream hydro power plants; sometimes in the summer and autumn the dam deflects all the water into this pipe (Fig. 11). Downstream the dam and due to the significant habitats modification, it appears as *Phoxinus phoxinus* is the dominant species, followed by *Salmo trutta fario* and accidentally *Barbus meridionalis* (Tab. 2). The CF-IBI score (27) reveals some expected species are rare and tolerant species as dominant.

On the O_{18} sector the river passes in a new relief unit type (Mateeşti and Sârbeşti hills area). The near upstream dam is a negative influence and downstream there is the appearance of more types of human impact categories (riverbed minerals overexploitation till the clay substratum (Figs. 12 and 13), affecting the macroinvertebrates food of fish; the pollution from mineral exploitations; roads presence in the riverbed (Fig. 14); and a two m high barrage (Fig. 15) with no fish leather which interrupt the fish populations connectivity), diminishing the river quality; till Ciupercenii de Olteţ locality CF-IBI value is only 2.

Figure 6: The Olteţ River valley at the entrance in the Polovragi Depression.

Figure 7: Natural lotic habitat in the upper sector of Polovragi Depression.

Table 2: The sampled fish material, in the lower trout zone area of Olteţ River (O_{14}-O_{18}); *Salmo trutta fario - S.t.f.*; *Phoxinus phoxinus - P.p.*; *Orthrias barbatulus - O.b.*; *Barbus meridionalis - B.m.*

Sampling stations	Sampling station localization and description	Species	Number of individuals	Abundance (%)
O_{14}	N - 45.2029 E - 23.7807 8 km downstrean the confluence of Olteţ and Ungurelul rivers. 200-300 m downstream the Cheile Olteţului Gorge. Riverbed length maximum 8 m and minimum 4 m. Maximum water depth 100 cm.	*S.t.f.*	66	100
O_{15}	N - 45.1836 E - 23.7859 9 km downstrean the confluence of Olteţ and Ungurelul rivers. 1 km downstream the Cheile Olteţului Gorge. Riverbed length maximum 8 m and minimum 4 m. Maximum water depth 100 cm.	*S.t.f.*	83	100
O_{16}	N - 45.1792 E - 23.7856 10 km downstrean the confluence of Olteţ and Ungurelul rivers. 2 km downstream the Cheile Olteţului Gorge. Riverbed length maximum 8 m and minimum 4 m. Maximum water depth 100 cm.	*O.b.*	5	100
O_{17}	N - 45.1555 E - 23.7905 1 km downstrean the dam and 10 km downstream the sonfluence of Olteţ and Ungurelul rivers. 3 km downstream the Cheile Olteţului Gorge. Riverbed length maximum 8 m and minimum 4 m. Maximum water depth 100 cm.	*S.t.f.* *P.p.* *B.m.*	23 55 1	29.11 69.62 1.26
O_{18}	N - 45.1387 E - 23.7931 2 km downstrean the dam and 11 km downstream the sonfluence of Olteţ and Ungurelul rivers. 4 km downstream the Cheile Olteţului Gorge. Riverbed length maximum 8 m and minimum 4 m. Maximum water depth 100 cm.	*B.m.*	3	100

Figure 8: Disturbed lotic habitat due to the local riverbed minerals chaotic exploitation.

Figure 9: Artificial cavity where few *Orthrias barbatulus* were sampled.

Figure 10: The dam fragmented this rivers sector lotic continuum.

Figure 11: Almost drought riverbed downstream the dam.

Figure 12: Clay river substratum with no more sand, gravels and boulders.

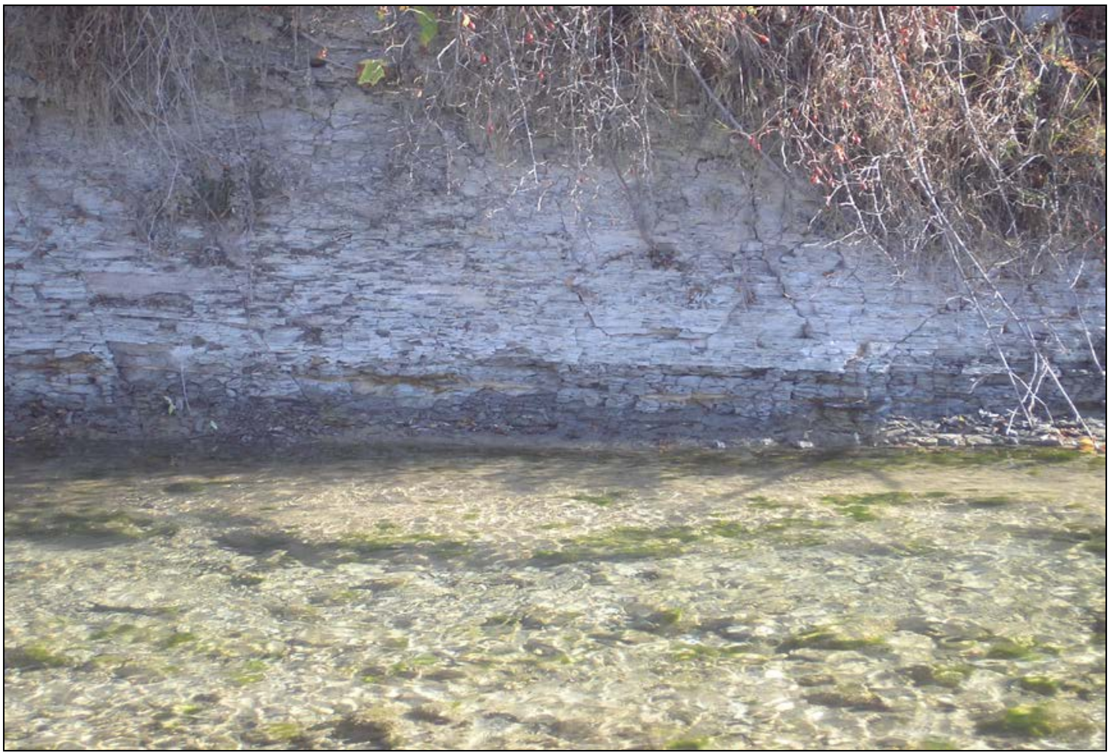

Figure 13: Clay substratum and erosion on riverbed after mineral overexploitation.

Figure 14: Road in the riverbed.

Figure 15: Concrete barrage with no fish leather.

A pre-Common nase zone is present. Due to complex, significant and continuous human impact on this depression area, the Grayling and Meridional Barbel ichthyological zone is not present. A lot of species characteristic for this ichthyological zone in Romanian Carpathians (Bănărescu, 1964) are missing (*Thymallus thymallus, Cottus gobio, Alburnoides bipunctatus, Cobitis romanica, Cobitis aurata*) and the present ones are there in short sectors and with a relatively low number of individuals (*Phoxinus phoxinus, Orthrias barbatulus* - accidental, *Barbus meridionalis* - accidental). The only three juveniles of *Barbus meridionalis* sampled in the lower sector of this depression area reveal also a very low ecological status of the ichthyofauna. This depression area fish fauna conservation status decreases significantly from its excellent status in its upper zone, to average/low in its middle zone, to very bad conservation status in its lower zone, due to the human impact effects.

❸ The **Podişul Getic - northern sector**, can be considered as being from the ichthyological point of view in majority to the Common nase zone as a continuity of the upper pre-Common nase zone, with the exception of the first sampling stations (O_{19}-O_{23}), which are in the Grayling and Mediterranean barbell zone influence, but also represent a transition zone to the next ichthyological zone; this is the first appearance of the *Alburnoides bipunctatus* and *Squalius cephalus* species. The upper diverse and high human impact presence induces an obvious fish fauna destructuring and a strong deviation of transition of ichthyofauna from the natural similar lotic sectors. After the recovering of all the water flow from the upstream two medium size power plants subterranean pipes, with a general substratum with boulders but also small patches with gravel, sand or even clay, the downstream lotic habitats can be considered as becoming again typical for this ichthyological zone, in conformity with Bănărescu zonation (1964). Excepting the *Chondrostoma nasus* whose absence is not clear as a natural one or rather induced by the human impact, the fish associations' structure reveal the presence of its ichthyological zone.

Table 3a: The sampled fish material, in the pre-Common nase zone area of Olteţ River (O_{19}-O_{23}); *Phoxinus phoxinus - P.p.*; *Barbus meridionalis - B.m.*; *Alburnoides bipunctatus - A.b.*; *Squalius cephalus - S.c.*; *Orthrias barbatulus - O.b.*; maximum riverbed length - max.r.l., minimum riverbed length - min.r.l., maximum water depth - max.w.d.

O_{19}	N - 45.1309, E - 23.7926, max.r.l. - 15 m, min.r.l. - 5.5 m, max.w.d. - 1.5 m S. 14 (Ciupercienii de Olteț aval de pragurile de beton)	*P.p.*	2	22.22%
		B.m.	7	77.77%
O_{20}	N - 45.1196, E - 23.7939, max.r.l. - 9 m, min.r.l. - 6 m, max.w.d. - 80 cm S. 15 (Sârbești)	*P.p.*	26	41.93%
		A.b.	34	54.83%
		B.m.	2	3.22%
O_{21}	N - 45.1029, E - 23.7979, max.r.l. - 9.5 m, min.r.l. - 5 m, max.w.d. - 74 cm S. 16 (Alimpești)	*S.c.*	2	20%
		P.p.	8	80%
O_{22}	N - 45.0836, E - 23.8006, max.r.l. - 11 m, min.r.l. - 5 m, max.w.d. - 63 cm S. 17 (Alimpești II)	*S.c.*	4	44.44%
		A.b.	5	55.55%
O_{23}	N - 45.0703, E - 23.8043, max.r.l. - 10 m, min.r.l. - 7 m, max.w.d. - 60 cm S. 18 (Nistorești)	*S.c.*	1	4.16%
		A.b.	20	83.33%
		B.m.	2	8.33%
		O.b.	1	4.16%

In O_{18} the significant upstream human impact are still visible in the low number of species and individuals, in contradiction with the passing of the river in a new geographical unit category (Mateeşti and Sârbeşti hills area) which should induce an increase in fish species number. In O_{19} the second station of this sector, the river quality is recovering and the upper Mediterranean barbel species are still present and dominant. Downstream, the Common nase zone characteristic fish associations structure reveal a medium human impact on the natural habitats (Fig. 16), due to lotic habitat's fragmentation (Figs. 17-19), industrial (coal exploitation) pollution and riverine localities waste waters pollution (Tabs. 3a, b). CF-IBI score vary between 30 and 24, reflecting some expected species to be absent or rare, omnivores and tolerant species as dominant. The passing of the river in the next hills area (Cerna and Amaradia hills) induce/increase/double the fish species number (Tab. 3b).

Table 3b: The sampled fish, in Common nase zone (O_{24}-O_{32}); *Barbus meridionalis - B.m.*; *Alburnoides bipunctatus - A.b.*; *Squalius cephalus - S.c.*; *Orthrias barbatulus - O.b.*; *Gobio uranoscopus - G.u.*; *Gobio gobio - G.g.*; *Sabanejewia romanica - S.r.*

O_{24}	N - 45.0489, E - 23.8053, max.r.l. - 11 m, min.r.l. - 8 m, max.w.d. - 1 m.S. 19 (Alun Dam)	*S.c.*	1	7.69%
		A.b.	7	53.84%
		B.m.	5	38.46%
O_{25}	N - 45.0359, E - 23.8158, max.r.l. - 14 m, min.r.l. - 7 m, max.w.d. - 60 cm S. 20 (Alun)	*S.c.*	1	10%
		A.b.	9	90%
O_{26}	N - 45.0049, E - 23.8227, max.r.l. - 12.5 m, min.r.l. - 7 m, max.w.d. - 54 cm S. 21 (Alun coal exploitation)	*S.c.*	5	15.15%
		A.b.	20	60.60%
		G.u.	1	3.03%
		B.m.	7	21.21%
O_{27}	N - 44.9823, E - 23.8247, max.r.l. - 10 m, min.r.l. - 4 m, max.w.d. - 90 cm S. 22 (Alun IV)	*A.b.*	24	72.72%
		G.u.	2	6.06%
		B.m.	7	21.21%
O_{28}	N - 44.9723, E - 23.8205, max.r.l. - 7.8 m, min.r.l. - 7.3 m, max.w.d. - 1 m S. 23 (Colţeşti)	*A.b.*	1	3.33%
		G.g.	1	3.33%
		G.u.	6	20%
		B.m.	10	33.33%
		S.r.	12	36.36%
O_{29}	N - 44.9432, E - 23.8349, max.r.l. - 10 m, min.r.l. - 3 m, max.w.d. - 40 cm S. 24 (Between Popești and Sinești)	*S.c.*	56	48.69%
		A.b.	11	9.56%
		G.u.	5	4.34%
		B.m.	13	11.30%
		O.b.	2	1.73%
		S.r.	28	24.34%
O_{30}	N - 44.9151, E - 23.8293, max.r.l. - 9 m, min.r.l. - 5 m, max.w.d. - 42 cm S. 25 (Urzica)	*Sc*	21	28.76%
		Ab	8	10.95%
		Gu	2	2.73%
		Bm	4	5.47%
		Sr	38	52.05%
O_{31}	N - 44.8811, E - 23.8327, max.r.l. - 15 m, min.r.l. - 6 m, max.w.d. - 1.5 m S. 26 (Grădiștea)	*Sc*	14	21.87%
		Ab	3	4.68%
		Gu	31	48.43%
		Bm	4	6.25%
		Sr	12	18.75%
O_{32}	N - 44.8485, E - 23.8359, max.r.l. - 13 m, min.r.l. - 6 m max.w.d. - 77 cm S. 27 (Tina)	*Sc*	34	35.78%
		Ab	21	22.10%
		Gu	34	35.78%
		Bm	2	2.10%
		Sr	4	4.21%

Figure 16: Natural habitat of the river in the Common nase area.

Figure 17: Stone steps which do not permit the fish to migrate.

Figure 18: Unnatural lentic habitat and sand accumulation due to a downstream dam.

Figure 19: Dam which creates upstream unnatural lentic sectors.

❹ Downstream, the river flows from the hills area (Amaradia and Cerna hills) to the plateau area (Beicăi and Teslui plateaus) passing the **Podişul Getic - southern sector**, and in this sector the ichthyofauna structure is shifting to the Barbel zone (Tab. 4a, b), in conformity with the Bănărescu ichthyological zonation (1964). CF-IBI score vary between 32 and 25, reflecting a decrease in the abundance of sensitive species, some expected species absent or rare, omnivores and tolerant species as dominant.

Table 4a: The sampled fish material, in the upper barbell zone area of Olteţ River (O_{33}-O_{40}); *Squalius cephalus - S.c.*; *Barbus meridionalis - B.m.*; *Alburnoides bipunctatus - A.b.*; *Gobio gobio - G.g.*; *Gobio uranoscopus - G.u.*; *Barbus barbus - B.b.*; *Sabanejewia romanica - S.r.*; *Alburnus alburnus - A.a.*

O_{33}	N - 44.8182, E - 23.8326, max.r.l. - 17 m, min.r.l. - 12 m, max.w.d. - 53 cm	*Sc*	5	12.82%
		Ab	4	10.25%
		Gg	1	2.56%
		Gu	22	56.41%
		Bb	1	2.56%
		Bm	5	12.82%
		Sr	1	2.56%
O_{34}	N - 44.7897, E - 23.8417, max.r.l. - 17.3 m, min.r.l. - 12 m, max.w.d. - 40 cm	*Sc*	12	34.28%
		Aa	1	2.85%
		Ab	9	25.71%
		Gg	1	2.85%
		Gu	9	25.71%
		Bm	1	2.85%
		Sr	2	5.71%
O_{35}	N - 44.7794, E - 23.8578, max.r.l. - 31.8 m, min.r.l. - 19.1 m, max.w.d. - 54 cm	*Sc*	15	37.5%
		Ab	10	25%
		Gu	13	32.5%
		Bm	1	2.5%
		Sr	1	2.5%
O_{36}	N - 44.7140, E - 23.9067, max.r.l. - 45.8 m, min.r.l. - 28.8 m, max.w.d. - 75.2 cm S.	*Sc*	32	51.61%
		Aa	8	12.90%
		Ab	5	8.06%
		Gu	15	24.19%
		Bm	2	3.22%
O_{37}	N - 44.6661, E - 23.9254, max.r.l. - 43 m, min.r.l. - 32.5 m, max.w.d. - 30 cm	*Sc*	68	45.94%
		Aa	1	0.67%
		Ab	55	37.16%
		Gu	15	10.13%
		Bm	5	3.37%
		Sr	4	2.70%
O_{38}	N - 44.6364, E - 23.9560, max.r.l. - 37 m, min.r.l. - 24 m, max.w.d. - 44 cm	*Sc*	32	76.19%
		Ab	6	14.28%
		Gg	1	2.38%
		Gu	1	2.38%
		Bm	1	2.38%
		Sr	1	2.38%

Table 4a (continuing): The sampled fish material, in the upper barbell zone area of Olteţ River (O_{39}-O_{40}); *Squalius cephalus - S.c.*; *Alburnoides bipunctatus - A.b.*; *Gobio gobio - G.g.*; *Gobio uranoscopus - G.u.*; *Sabanejewia romanica - S.r.*; *Alburnus alburnus - A.a.*

O_{39}	N - 44.6120, E - 23.9606, max.r.l. - 53.4 m, min.r.l. - 46 m, max.w.d. - 64 cm	*Sc*	44	83.01%
		Gg	1	1.88%
		Bm	4	7.54%
		Sr	4	7.54%
O_{40}	N - 44.5653, E - 23.9800, max.r.l. – 88.7 m, min.r.l. - 64 m, max.w.d. - 50 cm	*Sc*	33	73.33%
		Aa	1	2.22%
		Ab	4	8.88%
		Gu	1	2.22%
		Bm	4	8.88%
		Sr	2	4.44%

Table 4b: The sampled fish material, in the lower barbell zone area of Olteţ River (O_{41}-O_{43}); *Squalius cephalus - S.c.*; *Alburnoides bipunctatus - A.b.*; *Rhodeus sericeus amarus - R.s.a.*; *Carasius auratus gibelio - C.a.g.*; *Barbus barbus - B.b.*; *Sabanejewia romanica - S.r.*; *Sabanejewia aurata - S.a.*; *Pseudorasbora parva - P.pa.*; *Alburnus alburnus - A.a.*; *Romanogobio kessleri - R.k.*

O_{41}	N - 44.5338, E - 23.9996, max.r.l. - 61 m, min.r.l., max.w.d. - 61 cm	*Ab*	7	58.33%
		Rsa	2	12.66%
		Pp	1	8.33%
		Cag	2	12.66%
O_{42}	N - 44.5158, E - 24.0252, max.r.l. - 55 m, min.r.l., max.w.d. - 40 cm	*Sc*	73	35.96%
		Aa	1	0.49%
		Rsa	12	5.91%
		Rk	20	9.85%
		Bb	14	6.89%
		Sr	3	1.47%
		Sa	80	39.40%
O_{43}	N - 44.4845, E - 24.0806, max.r.l. - 60 m, min.r.l. - 28 m, max.w.d. - 50 cm	*Sc*	105	52.23%
		Aa	1	0.49%
		Rsa	1	0.49%
		Rk	25	12.43%
		Bb	6	2.98%
		Sa	63	31.34%

Table 4b (continuing): The sampled fish material, in the lower barbell zone area of Olteţ River (O_{44}-O_{47}); *Squalius cephalus - S.c.*; *Rhodeus sericeus amarus - R.s.a.*; *Carasius auratus gibelio - C.a.g.*; *Gobio gobio - G.g.*; *Barbus b - B.b.*; *Sabanejewia romanica - S.r.*; *Sabanejewia aurata - S.a.*; *Pseudorasbora parva - P.pa.*; *Romanogobio kessleri - R.k.*

O_{44}	N - 44.4611, E - 24.0951, max.r.l. - 40 m, min.r.l. - 21 m, max.w.d. - 40 cm	*Sc*	235	74.73%
		Rsa	3	0.82%
		Rk	25	6.88%
		Bb	16	4.40%
		Sr	1	0.27%
		Sa	83	22.86%
O_{45}	N - 44.4487, E - 24.1105, max.r.l. - 46 m; min.r.l. - 25 m, max.w.d. - 60 cm	*Sc*	180	64.05%
		Rsa	8	2.84%
		Gg	5	1.77%
		Rk	13	4.62%
		Bb	6	2.13%
		Cag	1	0.35%
		Sr	2	0.71%
		Sa	66	23.48%
O_{46}	N - 44.4208, E - 24.1069, max.r.l. - 60 cm, min.r.l. - 20 m, max.w.d. - 56 cm	*Sc*	263	78.74%
		Rsa	6	1.79%
		Gg	2	0.59%
		Rk	10	2.99%
		Bb	15	4.49%
		Sa	38	11.37%
O_{47}	N - 44.3936, E - 24.1121, max.r.l. - 46 m, min.r.l. - 32 m, max.w.d. - 60 cm	*Sc*	146	50%
		Rsa	16	5.47%
		Gg	5	1.71%
		Rk	3	1.02%
		Ppa	1	0.34%
		Bb	2	0.68%
		Cag	1	0.34%
		Sa	118	40.41%

The river passing from hills area to plateau area, the significant increasing of the Olteţ River water volume due to some important tributaries (Fig. 20) and the dominance of fine sand river bed sectors (Fig. 21) is the natural explanation for the local ichthyofauna shifting to the Barbel zone.

The deviations of the ichthyofauna structure from the natural Barbel zone one in the Romanian Carpathians (Bănărescu, 1964) in these sectors can be explained by the local human impacts like: pollution, habitat modifications (Fig. 23), roads in the riverbed and illegal fishing (Fig. 22).

Figure 20: Confluence of Olteţ River with Cerna River (Olteţ River biggest tributary).

Figure 21: The appearance of massive fine sandy riverbeds and banks areas.

Figure 22: Roads in the river.

Figure 23: River linearization using rockfill banks; near the Dinculeşti locality.

❺ Downstream the river flows in and passes (48-55 sampling stations) the **Câmpia Română/Romanian Plain sector**, (Caracal Plain and Olt River meadow) sector which has an ichthyofauna structure that is shifting to the Carp zone, in conformity with the Bănărescu ichthyological zonation (1964).

Table 5: The sampled fish material, in the Carp zone area of Olteţ River (O_{48}-O_{56}); *Squalius cephalus* - *S.c.*; *Rhodeus sericeus amarus* - *R.s.a.*; *Gobio gobio* - *G.g.*; *Romanogobio kesleri* - *R.k.*; *Barbus meridionalis* - *B.m.*; *Barbus barbus* - *B.b.*; *Carasius auratus gibelio* - *C.a.g.*; *Sabanejewia romanica* - *S.r.*; *Sabanejewia aurata* - *S.a.*; *Lepomis gibosus* - *L.g.*; *Alburnus alburnus* - *A.a.*; *Perca fluviatilis* - *P.f.*; *Pelecus cultratus* - *P.c.*; *Silurus glanis* - *S.g.*; 48-55 - quantitative samplings, 56 - qualitative samplings.

O_{48}	N - 44.3715, E - 24.1108, max.r.l. - 55 m, min.r.l. - 35 m, max.w.d. - 35 cm.	*Sc*	63	56.25%
		Rsa	9	8.03%
		Gg	15	13.39%
		Rk	8	7.14%
		Bm	1	0.89%
		Cag	1	0.89%
		Sr	1	0.89%
		Sa	8	7.14%
		Lg	2	1.78%
O_{49}	N - 44.3448, E - 24.1015, max.r.l. - 39 m, min.r.l. - 22 m, max.w.d. - 50 cm	*Sc*	62	50%
		Rsa	2	1.61%
		Gg	3	2.41%
		Rk	12	9.67%
		Bm	1	0.80%
		Cag	2	1.61%
		Sa	42	33.87%
O_{50}	N - 44.3207, E - 24.1217, max.r.l. - 60 m, min.r.l. - 39 m, max.w.d. - 55 cm.	*Sc*	5	3.75%
		Rsa	3	2.25%
		Gg	2	1.50%
		Rk	77	57.89%
		Cag	1	0.75%
		Sr	1	0.75%
		Sa	44	33.08%
O_{51}	N - 44.2855, E - 24.2213, max.r.l. - 72 m, min.r.l. - 35 m, max.w.d. - 55 cm.	*Sc*	48	31.78%
		Aa	1	0.66%
		Rsa	2	1.32%
		Gg	2	1.32%
		Rk	18	11.92%
		Bb	1	0.66%
		Bm	1	0.66%
		Cag	7	4.63%
		Sr	1	0.66%
		Sa	70	46.35%

O_{52}	N - 44.2844, E - 24.2446, max.r.l. - 66 m, min.r.l. - 25 m, max.w.d. - 60 cm	*Sc*	7	2.37%
		Rsa	1	0.33%
		Rk	51	17.28%
		Cag	1	0.33%
		Sa	235	79.66%
O_{53}	N - 44.2331, E - 24.3396, max.r.l. - 50 m, min.r.l. - 20 m, max.w.d. - 70 cm	*Sc*	42	33.07%
		Aa	1	0.78%
		Gg	2	1.57%
		Rk	40	31.49%
		Bm	1	0.78%
		Cag	5	3.93%
		Sa	36	28.34%
O_{54}	N - 44.2332, E - 24.3649, max.r.l. - 42 m, min.r.l. - 13 m, max.w.d. - 1.5 m	*Sc*	17	4.76%
		Aa	2	0.56%
		Ab	1	0.28%
		Rk	7	1.96%
		Cag	325	91.03%
		Sa	5	1.40%
O_{55}	N - 44.2296, E - 24.3978, max.r.l. - 47 m, min.r.l. - 24 m, max.w.d - 1 m	*Sc*	4	10%
		Aa	7	17.5%
		Rk	25	62.5%
		Cag	1	2.5%
		Lg	1	2.5%
		Ac	2	5%
O_{56}	N - 44.2231, E - 24.4359, max.r.l. - 95 m, min.r.l. - 80 m, max.w.d. - 2.5	*Sc*	3	2.14%
		Aa	17	12.14%
		Rk	15	10.71%
		Cc	1	0.71%
		Cag	99	70.71%
		Lg	3	2.14%
		Ac	2	1.42%
O_{56}	N - 44.2231, E - 24.4359, max.r.l. - 95 m, min.r.l. - 80 m, Maximum water depth - 2.5 m	*El*	+	+
		Abr	+	+
		Aa	+	+
		Rsa	+	+
		Ppa	+	+
		Pf	+	+
		Pc	+	+
		Sg	+	+

The high river water volume (Fig. 24), the presence of only fine sand river bed sectors (Fig. 26), the lowland aquatic plants areas, the slow water speed and the relatively meandered course are the natural explanation for the local fish fauna shifting to the carp zone.

The deviations of the ichthyofauna structure from the natural ones (Bănărescu, 1964) in these sectors can be explained by the local human impacts like: pollution, habitat modifications (Figs. 28 and 29), improper wastes management (Fig. 25) and illegal fishing.

CF-IBI score vary between 30 and 22, reflecting a decrease abundance of sensitive species, some expected species absent or rare, omnivores and tolerant species dominant.

Figure 24: Characteristic habitat in the lower sector of Olteţ River.

Figure 25: The constant presence of solid waste on the river banks.

Figure 26: Lower course of Olteţ River.

Figure 27: Direct polluted spills in the river in Balş urban area.

Figure 28: Olteţ River, four km from its confluence with Olt River.

Figure 29: Natural flow of Olteţ River at one km from the confluence with Olt River.

DISCUSSION

From the perspective of the local fish ichthyofauna associations, the O_{1-15} sector represents the upper trout zone in a relatively pristine area. O_{54-56} sections represent the most typical habitats for the plain macro habitats of Olteţ River, the exception of O_{55} sector is caused by the human impact in the locality Fălcoiu (O_{55}). $O_{16-17-21}$ has a close degree of similarity because the local fish ichthyocenoses react to the effects of the riverbed exploitations. $O_{50-52-55}$ has a close degree of similarity because the local fish ichthyocenoses react to a certain communal (Barza, Şopârlița and Fălcoiu) human impact in a low land macro habitats type. O_{1-13} is in a typically mountainous/Carpathian area and O_{14-18} is in a typically submountainous/subcarpathian zone, its lower part, O_{16-18} sector, being strongly influenced by the human impact. O_{18-19} has a close degree of similarity because the local fish ichthyocenoses react to the presence of concrete medium sized (two m) dams.

Using the graph (Fig. 30) where the interrupted columns represent the stations where the number of species increases from the previous station, the black columns represent the number of species that decrease from the previous station and the grey columns represent those stations that have the same number of species with the previous station; we can emphasize the fact that there are many variations in species number, either because of the basic natural/geographic causes or due to anthropogenic causes.

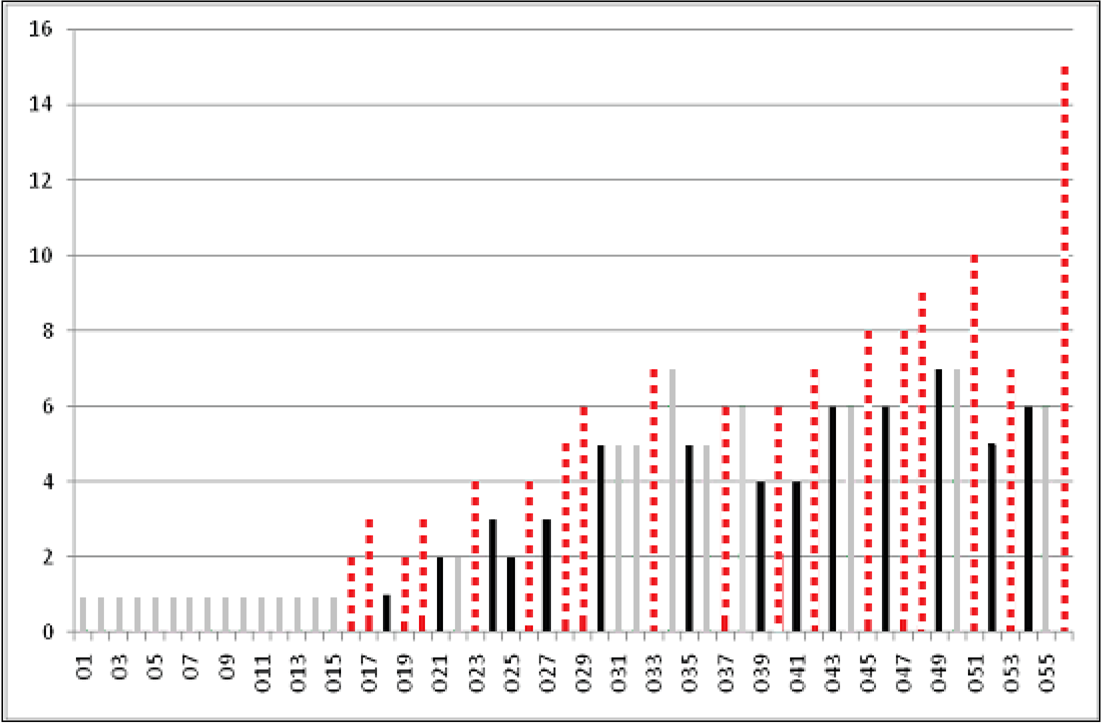

Figure 30: Fluctuations of fish species in each station on the course of Olteţ River.

The increasing of fish species number in 16-17, 19-20, 26, 29, 40, 47-48, 53, 56, sampling sectors reveal the natural changing of the ichthyological zone due to the river passing from one type of **geographical**/relief unit category to another category.

The unnatural decreasing of fish species number in the 18, 21, 24-25, 27, 30, 35, 39, 41, 43, 46, 49, 52, 54, sampling sectors have a **human** activities impact origin.

Other cases of river sectors (23, 28, 33, 37, 40, 42, 45, 51) where the fish species number increase without a connection with the geographical/relief units changing, is represented through **recovering** of the river habitats quality due to natural self-cleaning processes downstream of some anthropogenicaly impacted river sectors. It can be noticed that the recovery period, based on distances on the downstream sectors with problems, are faster in the upper ichthyological zones and slower in the lower ichthyological zones. This meaning that the fish communities' characteristics for the lower course of the river are easier to be negatively affected by the human impact than the upper one, at least in the Olteț River case. The slower self-cleaning capacity of the river and the much easier natural access of the humans to the river (localities, activities, etc.) can be explanations.

The fish communities' similarity in the 56 sampled lotic sectors along the Olteț River, based on the relative abundance of the component fish species, reveal the relation among the ichthyologic zones (Bănărescu, 1964) and some specific habitats presence, related also with significant different geographical units (Fig. 31).

In general, the passing of the river from one type of geographical/relief type unit to another induces the changing of the ichthyological zone and the increase of the fish species number. The cases where this situation did not appear are because different human impacts create unnatural variations in fish communities' compositions and individuals' number. It highlighted the fact that the geographical elements, human impact and the self-cleaning capacity of the river are the main driving forces which induce the fish fauna variability.

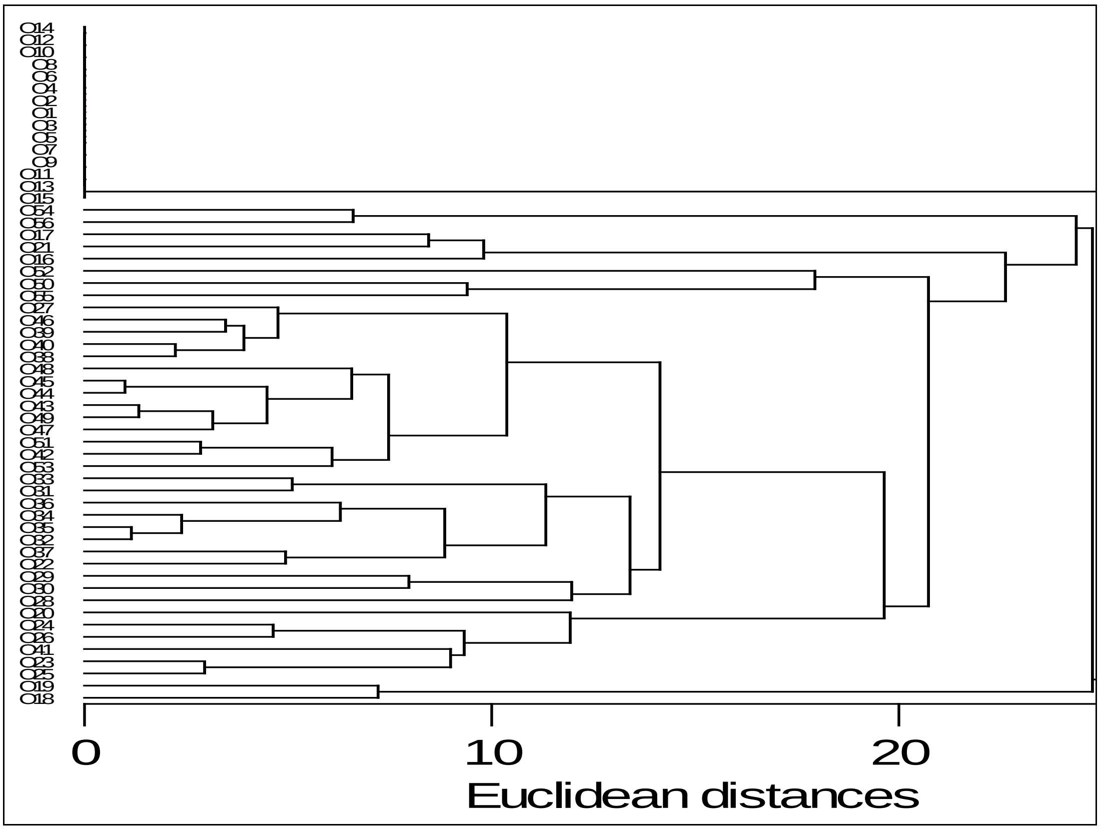

Figure 31: The fish communities' similarity along Olteț River, based on the component species relative abundance.

A half of the century which passes from the Bănărescu fish fauna study (1964) of the Olteț River lower sector, reveals significant modifications in ichthyofauna structure (Tab. 6) mainly due to significant habitat changes such as the creation of an important lenitic area at the Olteț-Olt confluence.

Table 6: Ichthyofauna (1960s/present) in the Balş city to the Olteţ-Olt rivers confluence sector.

	1960s	Present
1.	*Gobio kessleri*	*Gobio kessleri*
2.	*Sabanejewia aurata balcanica*	*Sabanejewia aurata*
3.	*Sabanejewia romanica*	*Sabanejewia romanica*
4.	*Gobio gobio*	*Gobio gobio*
5.	*Leuciscus cephalus*	*Leuciscus cephalus*
6.	*Carassius auratus gibelio*	*Carrasius auratus gibelio*
7.	*Barbus barbus*	*Barbus barbus*
8.	*Alburnus alburnus*	*Alburnus alburnus*
9.	*Exos lucius*	*Exos lucius*
10.	*Cyprinus carpio*	*Cyprinus carpio*
11.	*Cobitis taenia*	*Cobitis taenia*
12.	-	*Barbus meridionalis*
13.	-	*Rodeus sericeus amarus*
14.	-	*Alburnoides bipunctatus*
15.	-	*Acerina cernua*
16.	-	*Lepomis gibosus*
17.	-	*Abramis brama*
18.	-	*Aspius aspius*
19.	-	*Perca fluviatilis*
20.	-	*Pelecus cultratus*
21.	-	*Silurus glanis*

Despite the fact that more than half of a century has passed from the moment when Mr. Bănărescu M. P. proposed a zonation of the fish fauna on the Romanian national territory specific natural conditions (a zonation which is generally valuable even today), there were no other applied studies which reveal more than a relation between the fish associations and the major relief units (mountain, hills, plain) respectively to some gradients induced by the altitudinal variation. On the other hand, the Carpathian Fish Index of Biotic Integrity (CF-IBI), and other such biotic indexes, can highlight the fish communities' ecological state variation, but cannot explain it. This study highlighted the possibility to explain the fish fauna variation in relation with smaller relief units presence and variation, smaller relief units characterized by distinct geological and geomorphologic (biotope) conditions (even sometimes overlapped in their neighbouring areas) of the lotic sectors.

Naturally, there where the passing from one geographical/geological unit which appeared sharply, the ichthyological changes happened in relatively short sectors, and there where the biotopes characteristics changing gradually, gradually will appear the changes of ichthyofauna structure. In the last cases (e.g. Podişul Getic) it is more appropriate to work with ichthyological subzones, not only with ichthyological zones.

Even if the relation among geologic substrata (G) - relief units (R) - preponderant aquatic macro habitat types (A) - ichthyologic structures (F), (GRAF) is relatively obvious for Olteț River, the necessity of this type of approach through identifying the trends and gradients among these elements than rigid and arbitrary limiting separations along the river should be understood.

The degree of relativeness of the relations among geologic substrata - relief units - preponderant aquatic macro habitat types - ichthyologic structures (GRAF), increase in general under the increasing impact of many human impact categories, in these cases/sectors the interpretation of the fish fauna distribution and structure should be rather easy to interpretate.

The field effort (the relatively high number of sampling stations) necessary to such an approach is significantly higher than the majority of the fish inventory, assessment and monitoring types realized in the present Carpathians rivers.

In the less human influenced sectors, the ichthyologic classic zones (sensu Bănărescu 1964), have a better overlapping with the existent relief units which usually change together (mountain zones).

In the significant human influenced sectors, the ichthyologic classic zones (sensu Bănărescu 1964), have a relative overlapping with the existent relief units, the ichthyofauna changing are usually not immediate like in the mountainous zones (e.g. Polovraci Depression).

As far as the relief energy decrease, from high altitudes to low altitudes, the passing from one ichthyologic zone to the next one is far from the limit of one relief unit to the next one.

The natural passing from one ichthyologic zone to the next one can hardly be observed if the human impact is diversely present in those areas.

CONCLUSIONS

A white spot regarding the fish fauna of the upper and middle Olteț River was covered and the changes appeared in this respect and in the lower course were identified.

The passing of the river from one type of geographical/relief type unit to another induces the changing of the ichthyological zone and the increasing of the fish species number.

There where this natural situation did not appear is because different human impacts create unnatural variations in fish communities' compositions and individual numbers. The presence of various human impact, especially downstream the mountain area, make a relatively unbalanced fish fauna structure, in comparison with the mountainous sector.

Overall, the geographical and human impact elements, and a result of them, the self-cleaning capacity of the river, are the main driving forces which induce the fish fauna composition variability.

If such studies will be repeated in other Carpathian rivers, a new index of relative overlapping ichthyofauna-relief units categories which highlights and explains the missing/presence/importance of the human impact on fish fauna structure can be proposed.

ACKNOWLEDGMENTS

The authors would like to thank the colleague Nistorescu M. and the students Mărginean M., Stroilă V. and Răchită R. for their support in the field, and for the interesting professional moments spent together along the Olteţ River.

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THE DEAD SEA ECOSYSTEM INFLUENCED BY RED SEA – DEAD SEA CONDUIT PROJECT (PEACE CONDUIT)

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DOI: 10.2478/trser-2013-0019

KEYWORDS: Red Sea, Dead Sea, Peace Conduit, ecosystem impact, Jordan.

ABSTRACT

Recently, the rapid drying out of the Dead Sea is one of the most challenging problems facing the scientists and governments of the region. Its level has dropped more than 20 m in the past decade. Massive precipitation of halite from the water column has led to a decrease in Na^+ concentration, concomitant with an increase in Mg^{2+}, making the lake supersaturated with NaCl. This situation presents a big challenge to the microbial life of the lake. However, despite these harsh conditions in the lake, several microorganisms, including members of the groups bacteria, unicellular algae, fungi, viruses, and Archaea, have been able to survive.

To understand the factors that affect the microbes in the Dead Sea and to predict the possible effects of the planned conveyance of Red Sea water to the Dead Sea, a series of experiments were performed in the field, as well as in the laboratory. The results of the laboratory experiments showed that the growth of the *Dunaliella* was possible only when Dead Sea water was diluted with a minimum of 10% (by volume) Red Sea water. Addition of phosphate was essential for the algae to grow and growth rates and yields increased with increasing phosphate concentration and decreasing salinity. Field experiments revealed that the growth of algae was rapidly followed by the development of dense blooms of red halophilic Archaea. Although it should be realized that the closed system formed by the shallow ponds differs from the conditions in the lake, the results suggest that a microbial bloom, once formed, can remain present in the Dead Sea for months to years. These observations are important when attempting to predict how the biological properties of the lake may change in the future and they have important implications for the planning of the Red Sea-Dead Sea conduit.

The "Peace Conduit", a water carrier between the Red Sea and the Dead Sea, has been proposed to prevent the drying out of the lake and to restore the water level to a desired elevation. The present simulation experiments were designed at the Al Hussein bin Talal University (AHU) field station at Ma'an to get information on the ecological impacts of the Dead Sea when the "Peace Conduit" plans are implemented and massive quantities of Red Sea water will enter the Dead Sea and lower the salinity of the upper water layers.

Preliminary analysis was carried out of Fluorescence In Situ Hybridization (FISH) and Polar lipid of Archaea and Bacteria collected from the experimental ponds containing different Dead Sea-Red Sea water ratios, in order to analyze how they affect the microbial communities of the ponds. The Archaea community changed significantly according to the water mixture, presenting the greatest diversity when 30% Red Sea water was added to Dead Sea water.

ZUSAMMENFASSUNG: Das Ökosystem des Toten Meeres unter dem Einfluss des Roten Meeres - das Tote Meer Leitungsprojekt (Friedens-Leitung).

Neuerdings ist das rasche Austrocknen des Toten Meeres das herausforderndste Problem, dem die Wissenschaftler und Gouverneure gegenüberstehen. Sein Wasserspiegel sank im vergangenen Jahrzehnt um mehr als 20 m. Da der massive Halit Regen von der Wassersäule zu einer Verringerung der Konzentration von Na^+ mit einem gleichzeitigen Steigen von Mg^{2+} geführt hat, kommt es im Meer zu einer Übersättigung von NaCl. Diese Situation führte zu großen Schwierigkeiten für das mikrobielle Leben. Trotz dieser rauen Bedingungen haben einige Mikroorganismen, einschließlich aus dem Bereich der Bakterien, einzelligen Algen, Pilze, Viren and Archaea überlebt.

Um die Faktoren, die Mikroben im Toten Meer beeinträchtigen und die Auswirkungen der geplanten Verbindung zwischen dem Wasser des Roten Meeres mit dem Toten Meer zu verstehen, wurde sowohl im Freiland als auch im Labor eine Reihe von Experimenten durchgeführt. Die Ergebnisse der Laborexperimente zeigten, dass das Wachstum von *Dunaliella* nur dann möglich war, wenn das Wasser des Toten Meeres um Minimum 10% (entsprechend dem Volumen) mit Wasser des Roten Meeres verdünnt wurde. Die Zugabe von Phosphat war wesentlich für das Wachstum der Algen, wobei Wachstumsrate und Produktion mit steigender Phosphatkonzentration und abnehmender Salzkonzentration anstiegen. Die Freilanduntersuchungen zeigten, dass dem Wachstum der Algen rasch eine dichte Blüte der roten halophilen Archaea folgte. Obwohl man einsehen muss, dass das geschlossene Niedrigwassersystem der Teiche sich von den Bedingungen im Meer unterscheidet, zeigte sich, dass eine mikrobielle Blüte, einmal ausgebildet, sich längere Zeit über Monate bis zu Jahren im Toten Meer halten kann. Die Beobachtungen sind wichtig, wenn man sich anschickt vorauszusagen, wie sich die biologischen Eigenschaften des Meeres in Zukunft verändern könnten. Außerdem haben sie wichtige Konsequenzen für die Planung der Rohrleitung zwischen Rotem und Totem Meer.

Die “Frieden-Leitung”, der Bau einer Wasserleitung zwischen dem Roten und dem Toten Meer wurde vorgeschlagen, um das Austrocknen des Meeres zu verhindern und den Wasserstand in einer gewünschten Höhe wiederherzustellen. Die gegenwärtig durchgeführten, im Feldlabor der Al Hussein bin Talal Universität (AHU) geplanten Simulationsversuche, sollen Informationen liefern zu den ökologischen Auswirkungen auf das Tote Meer, wenn die Pläne der “Friedens-Leitung” umgesetzt werden und beträchtliche Mengen von Wasser aus dem Roten Meer in das Tote Meer fließen werden, wobei der Salzgehalt der oberen Wasserschichten gesenkt wird.

Die vorläufige durchgeführte Analyse der Fluoreszenz-In-Situ-Hybridisation (FISH) und der Polar-Lipede von Archaea sowie Bakterien, die aus den Experimentierungsteichen mit unterschiedlichem Anteil aus dem Roten Meer entnommenen Wassers beschickt wurden, zeigt, dass die Archaea Gemeinschaft sich je nach Wassergemisch signifikant verändert hat und dann die höchste Diversität aufweist, wenn 30% Wasser aus dem Roten Meer zu dem aus dem Toten Meer hinzugefügt wird.

REZUMAT: Influența proiectului „Conducta Marea Roșie-Marea Moartă” (Conducta Păcii) asupra ecosistemului Marea Moartă.

În ultimii ani, evaporarea rapidă a Mării Moarte este una dintre cele mai dificile probleme cu care se confruntă oamenii de știință și guvernele din regiune. Nivelul acesteia a scăzut cu peste 20 m în ultimii zece ani. Precipitările masive de halit din coloana de apă au dus la o creștere a concentrației ionilor de Na^+ concomitent cu creșterea concentrației ionilor de Mg^{2+}, ducând la suprasaturarea lacului cu NaCl. Această situație reprezintă o mare provocare

pentru viața microbiană a lacului. Cu toate acestea, în ciuda condițiilor dificile din lac, unele microorganisme, inclusiv membrii ai grupului Bacteria, alge unicelulare, fungi, viruși și arhee au reușit să supraviețuiască.

Pentru a înțelege factorii care au influențat microorganismele din Marea Moartă și pentru a putea prezice posibilele efecte ale aportului preconizat de apă din Marea Roșie în Marea Moartă, s-au efectuat o serie de experimente atât pe teren cât și în laborator. Rezultatele experimentelor în laborator au arătat că *Dunaliella* poate să se dezvolte numai la o diluție volumetrică a apei din Marea Moartă cu minim 10% apă din Marea Roșie. Adăugarea de fosfați a fost esențială pentru ca alga să se dezvolte, iar randamentele au crescut odată cu creșterea concentrației de fosfați și scăderea salinității. Experimentele de teren au arătat că dezvoltarea algelor a fost rapid urmată de înfloriri de mare densitate ale unor arhee halofile roșii. Deși trebuie luat în considerare faptul că sistemele închise formate de iazurile superficiale nu reproduc întocmai condițiile din Marea Moartă, rezultatele sugerează că înfloririle algale, odată formate, pot rămâne în Marea Moartă timp de luni și chiar ani de zile. Aceste observații sunt importante pentru încercarea de a prezice modul în care se pot modifica proprietățile biologice ale lacului și au implicații majore pentru planurile de construcție a conductei Marea Roșie - Marea Moartă.

Proiectul „Conducta Păcii”, construcția unui apeduct între Marea Roșie și Marea Moartă a fost propus pentru a preveni secarea lacului și pentru a readuce nivelul apei la cota dorită. Experimentele de simulare efectuate până în prezent au fost create la stațiunea de cercetări a Universității Al Hussein bin Talal (AHU), cu scopul de a testa informațiile cu privire la impactul ecologic asupra Mării Moarte după punerea în practică a planurilor pentru „Conducta Păcii”, atunci când cantități masive de apă din Marea Roșie vor intra în Marea Moartă și salinitatea straturilor superficiale va scădea.

Analizele preliminare au fost efectuate cu protocolul (FISH) - Fluorescence In Situ Hybridization și prin studiul lipidelor polare la arhee și bacterii colectate din iazurile experimentale ce conțin diferite procentaje de apă din Marea Moartă și Marea Roșie pentru a putea analiza modul în care aceste amestecuri afectează comunitățile microbiene din lacuri. Comunitățile de Archaea s-au modificat semnificativ odată cu schimbarea amestecului de apă, având cea mai mare diversitate la un procent de 30% apă din Marea Roșie adăugată în apă din Marea Moartă.

INTRODUCTION

The Dead Sea is a terminal lake located on the border between Jordan and Israel and it is part of a larger geological system known as the Jordan Rift valley. The lake consists of a deeper northern basin (deepest point at ~725 m below sea level) and a southern basin which has dried up and is being used for commercial mineral production. Until 1979 the Dead Sea was a meromictic lake with hypersaline, anoxic and sulphidic deep waters and a seasonally varying mixolimnion (Anati et al., 1987). The Jordan River and winter rain floods are the main sources of water input. Since the beginning of the 20th century the water budget of the Dead Sea has been negative, leading to a continuous decrease in Sea level (Anati et al., 1987; Oren, 2010). The extensive evaporation in the absence of major water sources led to an increase in the water density which caused the lake to overturn in 1979 (Steinhorn et al., 1979). Since then the Dead Sea remained holomictic and has been characterized by a constant state of NaCl supersaturation and halite deposition on the lake bottom. The decrease in Na^{+} ions has led to an increase in Mg^{++}, Ca^{++} and other dissolved ions.

During the 20th century, the Dead Sea level has dropped by more than 20 m and during the past decade, the level has dropped about one m per year on average (Gavrieli et al., 2002, 2005, 2005; Dvorkin et al., 2007). Severe problems affecting local infrastructure, tourism and industrial activities are caused by the phenomena (Oren et al., 2004). Due to the precipitation of halite, the total salt concentration has remained approximately constant at around 340 g l^{-1} and the pH at about six.

The increased salinity and the elevated concentration of bivalent ions make the Dead Sea too extreme and harsh environment to be tolerated by most organisms.

The life within the lake has been subjected to extensive research since the 1930s when Benjamin Elazari-Volcani (Wilkansky, 1936) isolated the first microorganisms from the sediments. Besides bacteria and Archaea, these isolates included algae (Elazari-Volcani, 1943 a), protozoa (Elazari-Volcani, 1943 b) and ciliates (Elazari-Volkani, 1944). Since then, several bacteria and Archaea isolates have been obtained in culture both from the sediment and from the water body (Oren, 2010). In comparison to other water bodies, the general abundance of organisms in the Dead Sea is very low. Two notable exceptions were two blooms in 1980 and 1992, when after severe winters, the upper meter of the water column was diluted by 15-30% and the cell concentration reached 20-35 x 10^6 cells/ml (Oren and Gurevich, 1995). The first metagenomic study on the planktonic community of the Dead Sea was recently conducted and showed Archaeal dominance in the water body (Bodaker et al., 2010). A comparison between the residual microbial community in the Dead Sea in 2007 and that followed the algal bloom in 2007 showed a marked differences between microbial communities.

Following rainy winters in 1980 and 1992, dense microbial blooms were observed in the Dead Sea. The formation of pycnocline at a depth varying between five and about 15 m was caused by the dilution of the upper water layers (Gavrieli et al., 1999). Oren et al. (1999 a, b), estimated that the algal density reached values up to 9×10^3 and 1.5×10^4 *Dunaliella* cells ml^{-1} in 1980 and 1992, respectively. Beside the algal blooms, red halophilic Archaea rapidly developed in high numbers; 2×10^7 and 3.5×10^7 Archaea ml^{-1} in 1980 and 1992, respectively.

These converted the entire lake to a red colour, and ended with the termination of the meromictic state and the renewed overturn of the water column (Oren and Anati, 1996). Not only is dilution a key point, but also the concentration of phosphate may have a main role as a limiting growth factor in the Dead Sea (Oren et al., 2004).

Since the last microbial bloom in the year 1992, and with the continuous drying out accompanied by a dramatic increase in the divalent/monovalent cation ratio, the lake has become ever more an extreme biotope (Bodaker et al., 2010).

As was demonstrated by Bodaker et al. (2010), the Dead Sea still supports the life of small Archaea and bacteria species communities, but conditions have probably become too extreme for active growth. *Dunaliella*, on the other hand, has not been seen in the water column during the past 12-13 years.

The planned water carrier between the Gulf of Aqaba (Red Sea) and the Dead Sea was proposed to minimize the drying out of the Dead Sea and to restore the water level to a desired situation. This idea has been discussed many times and the implementation of the project “Peace Conduit” could become real after the peace treaty between Jordan and Israel was signed in 1994 (Oren, 1999 a; Oren et al., 2004).

Peace conduit can be used for seawater desalination by using the reverse osmosis and the difference in elevation of about 416 m between the two seas. The formation of a stratified water column due to the dilution of the upper water layers of the Dead Sea resulted from the introduction of seawater from the Gulf of Aqaba (about 40 g/l salts), whether or not concentrated in the reverse osmosis process, into the Dead Sea (> 340 g/l total dissolved salts) (Oren et al., 2004).

This is a careful planned study on all aspects with positive and negative effects of the future implementation of the “Peace Conduit” regarding to the ecology of the lake. For this purpose, simulation pond experiments (Fig. 1) are being conducted on the grounds of the AHU, in experimental ponds (150 l) containing mixtures of Dead Sea water and Red Sea water (Fig. 2).

The ponds’ specific conditions were periodically altered to evaluate the effects on the microbial community. Some of the parameters studied were dependent on the mixing ratios of the water mixtures, enriched with low phosphate concentrations; total water volume were lowered naturally by evaporation or constantly maintained through inflow of fresh water.

Oren and his team (2004) showed that, when phosphate was provided, even a moderate dilution of the Dead Sea (with 15% Red Sea water) could give rise to extensive microbial blooms. Dramatic biological effects were observed in those ponds that had been filled with a mixture of 70% Dead Sea water and 30% Red Sea water. Algae and bacteria started to appear after 1.5-2 months even when no phosphate was added. The water in the ponds became highly turbid and red-brown coloured, mainly due to archaeal bacterioruberin pigments. In the experiment of Elevi Bardavid and his colleagues (2007 a), it has been observed that the microorganisms do not normally proliferate in the lake’s natural conditions, but the dilution of the Dead Sea may have dramatic effects on its microbial community.

The main goals of this research were to answer the following issues: the question of whether plankton that may enter the Dead Sea by the Peace Conduit contain available phosphate in concentrations sufficient to trigger algal blooms in the Dead Sea, a series of outdoor pond experiments were initiated at AHU in January 2011 in which different amounts of plankton collected from the Gulf of Aqaba were added to ponds filled with 80% Dead Sea water and 20% Red Sea water, to assess the simulating effects of a reduction in salinity by adding the necessary amount of seawater, thus allowing predictions of how the biological properties of the Dead Sea ecosystem will change in the future. Another purpose is to evaluate the environmental impact of the implementation of the Peace Conduit on the Dead Sea in the future.

MATERIALS AND METHODS

Experimental work

The open field-scale experiments, based on the mixtures of Dead Sea water and Red Sea water in different ratios are shown in table number 1, were incubated in experimental ponds on the grounds of the al Hussein bin Talal University (Fig. 1).

The experimental setup consisted of plastic tanks (150 l), which were buried in the ground at 75% of their heights (Fig. 2).

Figure 1: The location of the experimental ponds.

Figure 2: Tanks used for different mixtures of water.

These tanks were filled with 100 l of different mixtures of the Dead Sea water and water from the Red Sea (Gulf of Aqaba) as shown in table number 1.

Table 1: The proportions of Dead Sea and Red Sea (Gulf of Aqaba) waters in the water mixtures examined.

Pond number	Dead sea (%)	Red Sea (%)
1	100	0
2	90	10
3	90	10
4	90	10
5	85	15
6	85	15
7	85	15
8	70	30
9	70	30
10	70	30

The data of the present report were started in January 2011 and were based on ten ponds; each was amended with one µM KH_2PO_4 and inoculated with five ml of brine from a flask that had developed a bloom of *Dunaliella* and halophilic Archaea in a previous set of experiments.

The ponds were stirred daily and their water levels were kept constant by adding distilled water every one-two days, followed by thorough mixing. The ponds were then sampled for the content of algal chlorophyll and archaeal pigments once every week. Table 2 presents the ion concentration analyses of the samples withdrawn from the ponds.

Table 2: The ion concentration of the specific samples withdrawn from the studied ponds.

Concentration (ppm)					
Mixture of Dead Sea-Red Sea water	F	Cl	Br	(NO_3)	(SO_4)
100%	358	172095	4999	ND	172
90%	335	166434	3762	ND	205
85%	234	148572	3190	ND	352
80%	241	148131	3378	ND	215

Laboratory-scale experiments of microbial growth in the Dead Sea – Red Sea water mixtures

To investigate in depth the effect of salinity and phosphate concentration on the growth of *Dunaliella* in the Dead Sea - Red Sea waters, a series of lab experiments were set up, in which 100 ml Erlenmeyer flasks were filled with 75 ml of mixtures of Dead Sea water and filtered Red Sea water at different ratios based on table 1. All flasks were inoculated with a culture of *Dunaliella* and incubated at 30°C under constant illumination by white fluorescent tubes. To prevent evaporation and to ensure constant salinity for the duration of the experiment, the flasks were closed with screwed cork. After 25 days samples were taken for the chlorophyll assay.

Pigments determinations

The chlorophyll and other pigment contents in the experimental ponds were determined by filtering 50 ml of the sample volume through glass fibber filters (Whatman GF/C, 47 mm diameter) within hours after sampling. Filters were kept at -10^oC in the dark until further processing within three-four weeks. Filters were then extracted overnight in five ml methanol/acetone (1:1, by volume). For the determination of chlorophyll in laboratory cultures, 30 ml volume of liquid were filtered through 25 mm diameter GF/C filters and the filters were extracted in 2.5 ml of methanol/acetone. The extracts were cleared of particles by centrifugation and their absorption spectra (400-700 nm) was measured by spectrophotometer, using the solvent as a blank. Chlorophyll concentrations were calculated based on a specific absorption of 73.5 l mg^{-1} cm^{-1} for chlorophyll *a* at 665 nm.

Archaeal bacterioruberin pigments were quantified based on a specific absorption of 25.4 l mg^{-1} cm^{-1} at 496 nm for α-bacterioruberin. A correction was made for the contribution of algal pigments to the total absorbance at this wavelength, as outlined in Oren et al. (2004).

Microbial Diversity

FISH (Fluorescence in Situ hybridization)

FISH technique was used to avoid the need for cultivating a microorganism to recognize its presence and measure its distribution in a community. The microbial ecologists believe that there is potential to use molecular methods to understand microbial diversity. 16S rRNA sequences were used not only to permit culture-independent detection of population but also provide a means of observing pattern of their evolution.

Samples were collected from 85% of the Dead Sea and Red Sea mixture pond for hybridization experiments. Fixed samples were immobilized on glass slides by air drying. In situ hybridization was performed at 46°C for 90 min in a hybridization buffer containing 0.9 M NaCl, 20 mM Tris-HCl (pH 7) and 1% SDS. Probe concentrations were 5 nmol/µl. Probe EUB I (5'- FAM GCT GCC TCC CGT AGG AGT-3') and probe EUB II (5'-FAM GCA GCC ACC CGT AGG TGT-3') and EUB III (5'-FAM GCT GCC ACC CGT AGG TGT-3') were specifically for Eubacteria. For α-proteobacteria we used probe (5'-LYS GGT AAG GTT CTG CGC GTT-3') and Arch. 915 probe is specific for Archea (5'-CAL160 GTC CTC CCC CGCCAA TTC CT-3'). Hybridization mixtures were removed and the slides were washed for 15 min in buffer containing 20 mM Tris-HCl, a millimolar concentration of NaCl and 0.01% SDS at 48°C. The slides were examined under an epifluorescence microscope using different filters.

Fatty Acids Analysis

To understand the microbial communities, many researchers considered biomarkers as good indicator for this purpose. In this work the fatty acid composition of the lipids extracted from the 85% Dead Sea and Red Sea mixture pond was performed in an attempt to further characterize the microbial community and to define the presence of fatty acids that may be used as biomarkers for specific groups of bacteria.

The fatty acid methyl esters (FAME) were extracted with 1.25 ml hexane-methyl-tert-butyl-ether (1:1, v:v). The tubes were rotated for ten minutes, after that the upper phase including the FAME was transferred to new tubes. Then the FAME were analyzed on HP G1800B GCMS using an HP-5-MS column, the initial temperature being 120°C increasing at a rate of 5°C per min up to 240°C and then at a rate of 15°C per min up to 300°C for 20 min. FAME were identified on the basis of their equivalent chain length.

RESULTS

Field-scale experiments of microbial growth in the Dead Sea-Red Sea water mixtures

The field experiments showed that once a microbial bloom has formed in a Dead Sea-Red Sea water mixture, it may continue for a long time.

A bloom of *Dunaliella* and halophilic Archaea in a mixture of Dead Sea water and Red Sea water (Tab. 1) supplemented with 1 µM orthophosphate is expressed as a pigment concentrations (Figs. 3 and 4).

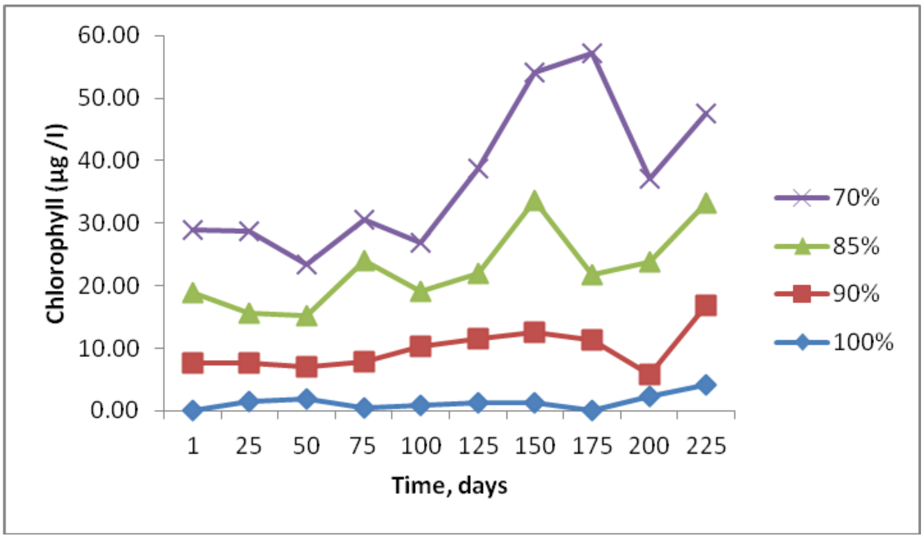

Figure 3: Algal bloom of *Dunaliella* in a mixture of different ratios of Dead Sea water and Red Sea water expressed as chlorophyll.

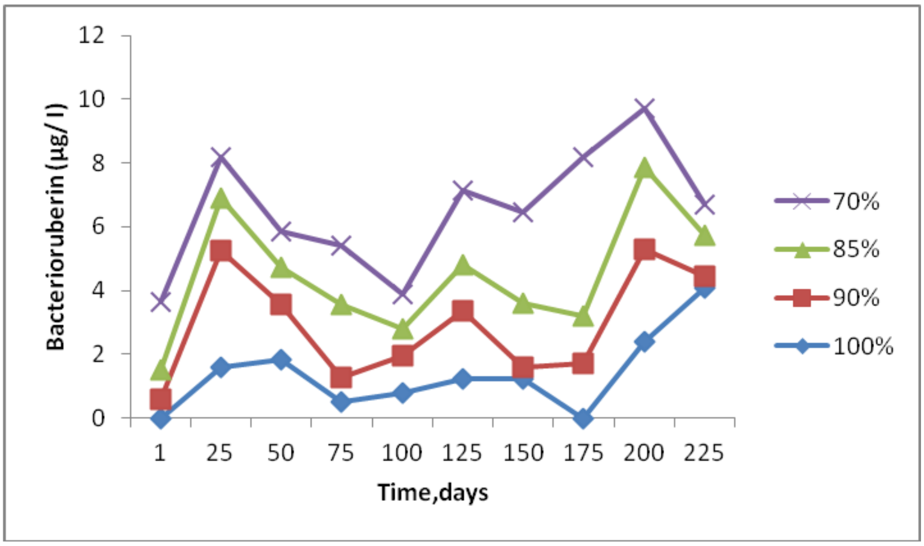

Figure 4: Halophilic Archaea bloom in a mixture of different ratios Dead Sea water and Red Sea water expressed as the concentration of bacterioruberin.

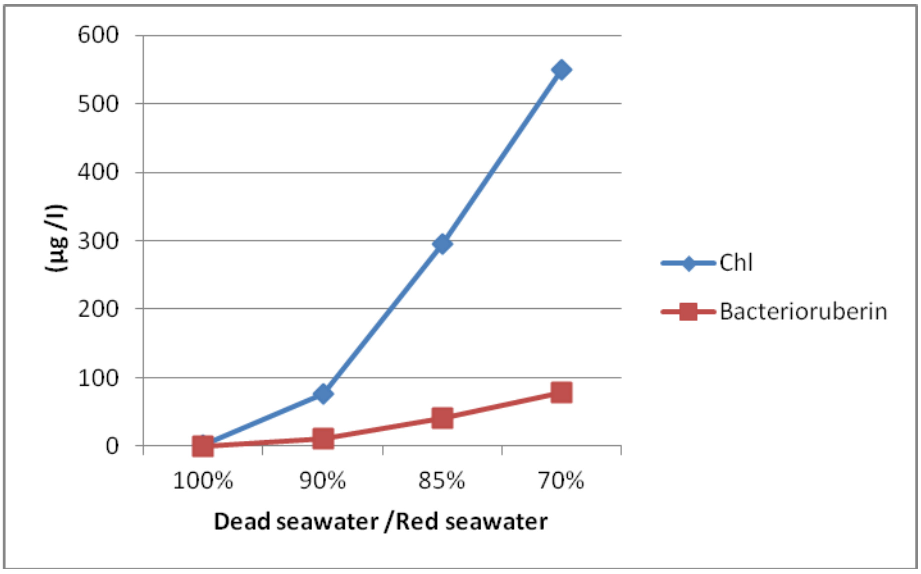

Figure 5: Concentrations of chlorophyll and Bacterioruberin (µg/L)
30 days time period.

In some of the ponds the dense biological communities developed within one-two months and the greatest extent of the algal and archaeal blooms was reached. These blooms depended on the degree of dilution and on the phosphate added. There is no significant biological growth in the 100% Dead Sea water pond, while very little occurred in mixtures of 90% Dead Sea water and 10% Red Sea water while clear cut growth in others mixtures. Algal pigment concentration reached values of up to 35.35 μg^{-1} chlorophyll liter after six months 70% mixture, which started to appear after one-two months. Mass development of algae was followed by the growth of halophilic Archaea, which are heterotrophic microorganisms that develop at the expense of organic compounds produced by the autotrophic algae. The bacterioruberin's halophilic archaea (reach up to 1.8 µg/l) imparted an intensely red color to the brine. The water in the ponds becomes highly turbid, and the algal chlorophyll and the archaeal bacterioruberin pigments confer brownish-green color to the water.

Laboratory - scale experiments of microbial growth in Dead Sea – Red Sea water mixtures

The results of this laboratory experiment study, in which different types of mixtures were supplemented with different concentrations of orthophosphate, showed an inoculum of *Dunaliella* and halophilic archaea after incubation in the light for four weeks. Algae developed only when the concentration of Dead Sea water in the mixtures was below 90%, and the rate at which the cells multiplied increased with decreasing salinity of the water mixture. The extent of the algal growth obtained was a function of the concentration of phosphate added in the laboratory experiment (Fig. 5). No further changes were noted when incubation was continued for three additional weeks. These results confirm open field experiments.

In Situ Hybridization Analysis of the samples

The field experiment samples were first analyzed by DAPI staining and in Situ Hybirdization. The preliminary results are shown in figure 6a-d.

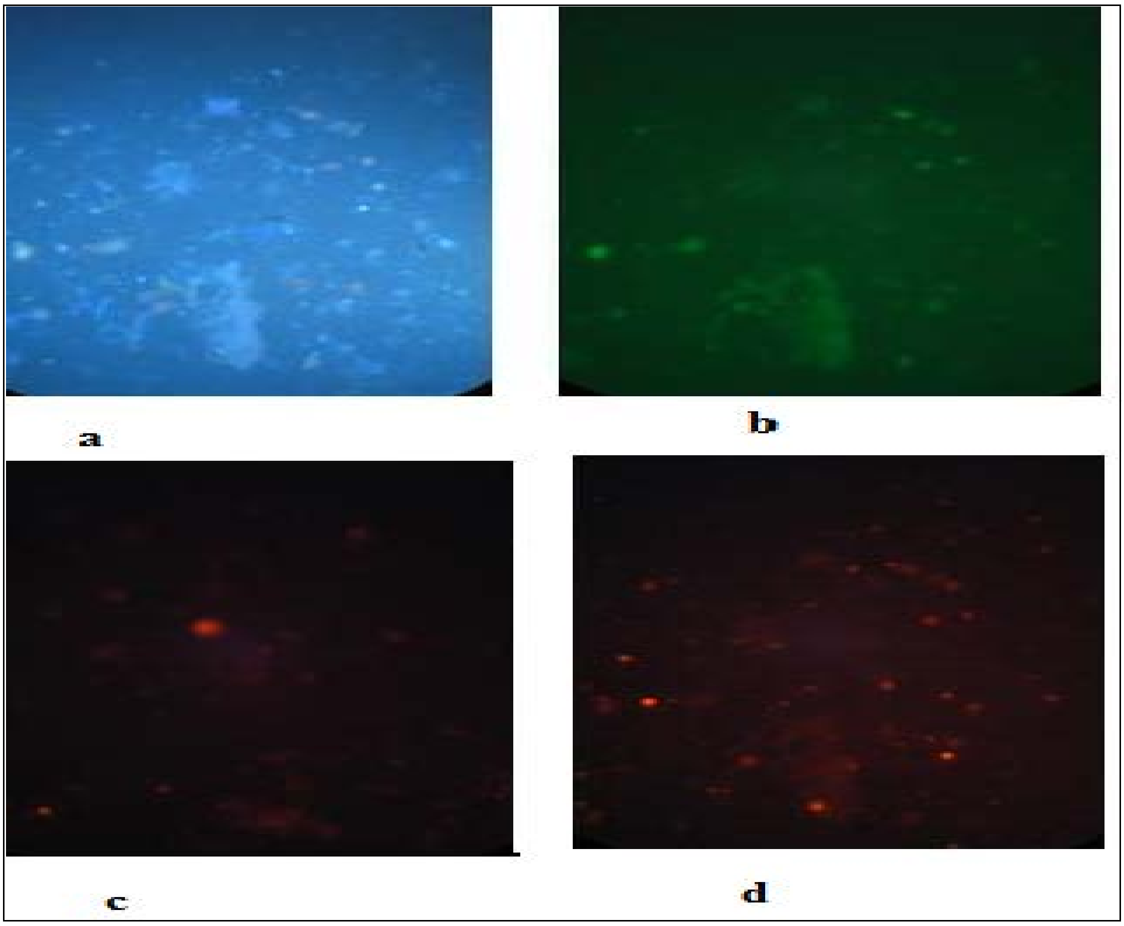

Figure 6 a-d: DAPI stain and hybridization signals for the same fields obtained with probes (EUB I, II, III, α-proteobacteria and Arc915);
a - DAPI, b - Eubacteria, c - α-Proteobacteria, d - Archaea.

The signals obtained with all oligonucleotide probes were strong and indicated high cellular rRNA content. The community was dominated by bacteria which were detected by the different probes used. Archea were also present.

Fatty Acids Analysis

Fatty acids are excellent biomarkers for specific microorganisms and their fatty acids signature can be recognized in complex lipid mixtures derived from microbial communities. Table 3 shows the fatty acids composition (mol% of total fatty acids extracted) of the different samples.

The fatty acids can serve as fingerprints of special microbial groups and can be used as a taxonomy signature for bacteria classification. They also carry information of the community structure. Fatty acids are valuable phenotypic indicators for characterizing pure culture. However, they often represent multiple-sources of environmental samples.

The preliminary analysis of the fatty acid methyl esters (FAME) composition in the mixture samples were dominated by 14:0, 15:0, 16:0, 17:0 and 18:0 (Tab. 3). Iso - and antiso - fatty acids (i.e., 15:0 iso, 15:0 antiso, 17:0 iso and 17:0 antiso) were major component of the total FAME (Tab. 3).

Table 3: Fatty acids composition (mol% of total fatty acids extracted) of the different mixture samples.

Fame	S1	S2	S3	S4	S5
14:0 methyl/14:0 iso	0.9	0.6	0.5	0.8	0.5
14:0	1.3	0.8	0.5	0.7	1.0
15:0 iso	30.9	29.6	30.2	27.5	45.7
15:0 anteiso	3.6	9.8	2.7	2.4	3.0
15:0	1.0		0.6	0.9	0.6
16:0 methyl/16:0 iso	15.7	8.1	16.7	20.7	17.8
16:0	10.6	8.8	8.6	9.2	12.6
17:0 iso	17.9	25.5	26.9	26.0	4.4
17:0 anteiso	8.4	7.8	11.0	7.7	12.9
17:0	0.6		0.4	0.6	
18:0 methyl/18:0 iso		0.8	0.4	0.6	0.5
18:1 cis 9	2.1	0.9	0.3	0.7	
18:1 cis 11	3.6	0.5			
18:0	3.5	3.3	1.2	1.4	1.0

As mentioned earlier, the analysis of fatty acids has been proven to be a valuable tool in the characterization of microbial communities, structures, nutritional status, metabolic activities and environmental stresses.

DISCUSSION

For many reasons, the Dead Sea was considered to be a hostile environment to life, being a far more extreme environment for life as compared to all other aquatic environments, particularly due to the higher salinity. Animals, plants and protozoa are absent. The primary producer *Dunaliella* and several species of halophilic Archaea may be found in this type of harsh environment (Oren, 1988). The general patterns of biological activity that occur in the aerobic water column of the Dead Sea and some of the interactive relationships between the biota are represented in figures 4 and 5.

Dunaliella is one of the most salt-adapted algae known so far, which is why its bloom is associated with the development of Archaea, which use the expanses of organic material produced by algae under the favourable diluted condition. The results of this report confirmed the earlier findings of other authors (Oren and Gurevich, 1995), which were noticed after the rainy winters of 1980 and 1992 which were showed in simulation experiments (Figs. 4 and 5). *Dunaliella* produced glycerol, which is one of the organic compounds that provide osmotic stabilization. The halophilic Archaea used the glycerol as nutrient source. Dilution of the Dead Sea water by 30% Red Sea water caused the development of algal and archaeal bloom that impart a strong greenish-brown colour. The algal and archaeal densities exceeded any biological blooms that had been witnessed so far in the lake.

The bioavailability of phosphate is accounted as a critical factor for the growth of microbial blooms in the lake. Dry and wet depositions are important sources of phosphate that enters the Dead Sea, in addition the direct input by the Jordan River and winter rain floods. Inorganic nitrogen is available in high contents as ammonium ions (Nissenbaum et al., 1990; Stiller and Nissenbaum, 1999). The concentration of dissolved phosphate in the Dead Sea was estimated by Stiller and Nissenbaum (1999) and Nissenbaum et al. (1990) of about one M. The determination of these ions' concentration is difficult due to the high molar concentration of salt. Our results from both indoor and outdoor simulation experiments demonstrated that the *Dunaliella* community highlight the important roles of phosphate as a key nutrient controlling the ecology of the Dead Sea (Figs. 4 and 5). Oren and Gurevich (1995), explained that any addition of new phosphate could enhance the algal growth and give chance for renewed growth of algal by formation of cyst-like structures, possibly zygotes, which sank to the bottom. Evidence showed that such thick-walled cysts serve as the inoculum that enables the rapid development of *Dunaliella* in the Dead Sea as soon as the upper water layers become diluted by freshwater floods.

As revealed by preliminary FISH analyses, bacteria and Archaea related to both Eubacteria and α-protobacteria also form an important part of prokaryotic community inhabiting the Dead Sea. Among the interesting characteristics of the organism is the haloadaptation mechanism used to enable life under hypersaline conditions. All the halophilic and halotolerant aerobic bacteria characterized until 2002 produce and/or accumulate organic "compatible" solutes such as ectoine, glycine betaine, and other solutes, to provide the necessary osmotic balance. Synthesis and degradation of those solutes can be regulated according to the extracellular salt concentration, enabling a considerable degree of adaptability to changes in the salinity of the medium (Oren, 1999 b).

Aerobic halophilic archaea accumulate potassium chloride in molar concentrations (Lanyi, 1974; Oren, 1999b). This strategy requires adaptations to intracellular processes to be functional at high salt concentrations. Proteins of the *Halobacteriales* are typically rich in acidic amino-acids, depleted of basic amino-acids, and relatively poor in hydrophobic amino acids. Such proteins generally require the presence of high salt concentrations for stability and activity (Lanyi, 1974). Accordingly, the microorganisms that possess these are unable to adapt to life below a (generally very high) minimal salt concentration. A similar strategy of adaptation to high salt was found in the obligatory anaerobic bacteria of *Halanaerobiales* order, phylogenetically affiliated with the low G+C branch of the *Firmicutes* (Oren, 1986; Oren, 1999 b). Another interesting feature of this microorganism is the presence of two sulfonolipids in its membrane composition (Baronio et al., 2010). It is known that the modification of membrane lipid composition is an important aspect of haloadaptation, preserving membrane integrity and function at high salt concentrations.

Results from this study suggest that the lipid may be used as a chemotaxonomic marker for the detection of groups within the halophilic microbial community in saltern ponds and other hypersaline environments. Our results have recently shown that lipid analysis of the biomass lipid profile is a powerful experimental approach to obtain information on the types of halophilic microorganisms that inhabit brines. As glycolipids of extremely halophilic archaea may serve as chemotaxonomic markers for the classification of these organisms. Therefore, our conclusion is that the novel lipid may represent a useful biomarker to obtain both qualitative and quantitative information about the distribution of this genus in hypersaline environments.

It is also interesting to understand the ecology of *Salinibacter* and the role it plays in the microbial community. Therefore, glycerol can be expected as one of the main nutrients available in hypersaline environments. It is produced in large quantities by the unicellular algae *Dunaliella*, which is the main or only primary producer in those habitats, including the experimental ponds. The use of glycerol by members of the *Halobacteriaceae* and *Salinibacter* had been demonstrated, and the overflow product formed by the latter was later identified (Elevi et al., 2007 b).

The experiments with FISH from the experimental ponds, using universal primers for Archaea, showed that the community's diversity will significantly change according to the amount of Red Sea water poured into the hypersaline lake. The greatest diversity was observed when the Red Sea water represented 30% of the water mixture. The sample containing the smaller number of bands was that containing 80% Dead Sea water and 20% Red Sea water, probably because only a small number of species can tolerate such a high salinity. It is important to stress that, although Dead Sea-Red Sea mixtures at other ratios presented less diversity, it doesn't mean they were less populated; it is just an indication of the variety of species present, not of the total number of cells. Finally, the present team believed that if the Peace Conduit is to be implemented, changes in the archaeal community of the Dead Sea are expected.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Alosufi A. for helping with the analyses, to biological department, Al Hussein Bin Talal University and for the financial support of the Scientific Research Support Fund, project no. S1/1/2009.

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DE GRUYTER OPEN

CLIMATIC INFLUENCE ON THE PHYTOPLANKTON COMMUNITIES OF THE UPPER REACHES OF THE SOUTHERN BUG RIVER (UKRAINE)

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DOI: 10.2478/trser-2013-0020

KEYWORDS: algae, lotic systems, seasonal changes, bio-indication, monitoring.

ABSTRACT

A total of 98 taxa of algae were observed in phytoplankton, sampled monthly, from the Khmelnitsky monitoring station in the Southern Bug River, Ukraine, between April 2010 and March 2011. Chlorophyta species are the richest taxonomic group with 46 taxa, followed by Bacillariophyta, Euglenophyta, Cyanoprokaryota, Dinophyta, Chrysophyta, Streptophyta, and Xanthophyta. Seasonal dynamics of species distribution in taxonomic divisions shows that the role of Bacillariophyta in communities was high in January-March, which were replaced by greens in March-September. Euglenoids were developed in February-December and blue-green algae in summer communities only. Strong positive correlations between temperature and species richness was observed. Abundance and biovolume of phytoplankton were maximal in summer, caused mostly by *Dolichospermum flos-aquae* (Lyngb.) Wacklin, Hoffmann and Komarek and *Ceratium hirundinella* (O. Müll.) Bergh. The river ecosystem has two periods of trophic levels - high at summer and low at winter. Bioindication characterizes the river as low alkaline and low mineralized with a moderate organic pollution level, revealed aspects of seasonal changes and revealed the main source of organic pollution as flowing from the catchment area during ice melting and rains. Organic pollution indices fluctuate within narrow limits suggesting relative stability of the river ecosystem that is shown also by Shannon indices. The calculated indices, comparative statistics, CCA, and bio-indication analysis exhibits a low pollution level in the Khmelnitsky monitoring station that can be used as a model of aquatic community dynamics under seasonal fluctuation in the southern boreal province climate, applicable for monitoring of the Southern Bug River.

ZUSAMMENFASSUNG: Der Einfluss des Klimas auf das Phytoplankton im Oberlauf des Bug-Flusses (Ukraine).

Am Oberlauf des Südlichen Bug, Ukraine, wurden an den monatlich beprobten Stellen im Monitoringgebiet Khmelnitsky zwischen April 2010 und März 2011 insgesamt 98 Taxa von phytoplanktonischen Algen festgestellt. Die Chlorophyten sind mit 46 systematischen Einheiten die reichste Gruppe, gefolgt von Arten der Bacillariophyta, Euglenophyta, Cyanoprokaryota, Dinophyta, Chrysophyta, Streptophyta und der Xanthophyta. Die jahreszeitliche Dynamik der Artenverteilung auf die taxonomischen Gruppen zeigen, dass während der Monate Januar bis März der Anteil der Bacillariophyta in den Gemeinschaften hoch war und die dann zwischen März und September von Grünalgen abgelöst wurden. Die

Euglenophyta waren von Februar bis Dezember in den Proben vorhanden, während die Blau-Grünalgen allein in den Sommergemeinschaften vorkamen. Es wurden enge positive Beziehungen zwischen Temperatur und Abundanz der Arten festgestellt. Abundanz und Biovolumen des Phytoplanktons zeigten während des Sommers Höchstwerte, die größtenteils bedingt waren durch das hohe Aufkommen von *Dolichospermum flos-aquae* (Lyngb.) Wacklin, Hoffmann and Komarek und *Ceratium hirundinella* (O. Müll.) Bergh. Das Ökosystem des Flusses hat zwei trophische Ebenen, eine hohe während des Sommers und eine niedrige während der Winterzeit. Anhand der Bioindikatoren wurde der Fluss im alkalinen Bereich, einer niedrigen Mineralisierung sowie einer moderaten organischen Belastung eingestuft und zeigte auch Aspekte der jahreszeitlichen Veränderungen. Außerdem konnte die Hauptquelle der organischen Belastung festgestellt werden, die sich während der Schneeschmelze und Regenwasser aus dem Einzugsgebiet sammelte. Die Indikatoren der organischen Belastung schwankten in engen Grenzen und wiesen dadurch auf eine relative Stabilität des Flussökosystems hin, die auch durch den Shannon-Index verdeutlicht wird. Die berechneten Indices, die vergleichenden statistischen Angaben, CCA sowie die Analyse der Bioindikatoren belegen eine niedrige Belastung im Beprobungsgebiet von Khmelnitsky, das als Beispiel für die Dynamik der aquatischen Gemeinschaften unter dem Einfluss jahreszeitlicher Schwankungen im Klimagebiet der südlichen borealen Provinz angesehen und für das Monitoring des Südlichen Bug verwendet werden kann.

REZUMAT: Influența climatică asupra comunităților fitoplanctonice din cursul superior al râului Bug de sud, Ucraina.

S-au observat un total de 98 taxoni de alge în fitoplanctonul studiat, pe baza unor eșantioane lunare, prelevate la stațiile de monitorizare Khmelnitsky în râul Bug, Ucraina, între aprilie 2010 și martie 2011. Chlorophyta reprezintă cel mai bogat grup taxonomic cu 46 taxoni, urmat de Bacillariophyta, Euglenophyta, Cyanoprokaryota, Dinophyta, Chrysophyta, Streptophyta și Xanthophyta. Dinamica sezonieră a distribuției speciilor în diviziunile taxonomice arată că rolul Bacillariofitelor în comunități a fost ridicat în ianuarie-martie, fiind înlocuite cu alge verzi în martie-septembrie. Euglenophyta au fost observate în probele din februarie-decembrie și algele albastre-verzi doar în comunitățile de vară. Au fost observate strânse corelații pozitive între temperatură și abundența speciilor. Abundența și biovolumul fitoplanctonului au avut valori maxime în timpul verii, fiind cauzate în cea mai mare parte de *Dolichospermum flos-aquae* (Lyngb.) Wacklin, Hoffmann şi Komarek şi *Ceratium hirundinella* (O. Müll.) Bergh. Ecosistemul râului are două perioade de nivele trofice, ridicate vara şi scăzute în timpul iernii. Cu ajutorul bioindicatorilor râul se prezintă la un nivel alcalin, mineralizare joasă şi de poluare organică moderată, relevându-se aspecte ale schimbărilor sezoniere. De asemenea, s-a putut depista principala sursă de poluare organică, care se colectează din bazinul hidrografic în timpul topirii gheții și a ploilor. Indicii de poluare organică fluctuează în limite înguste sugerând o stabilitate relativă a ecosistemului râului, care se arată, de asemenea, prin indicii Shannon. Indicii calculați, statisticile comparative, CCA și analizele de bio-indicatori scot în evidenţă un nivel scăzut de poluare, în stația de monitorizare Khmelnitsky, care poate fi folosit ca un model pentru dinamica comunităților acvatice sub influența fluctuației sezoniere în climatul sudului provinciei boreale, aplicabil pentru monitorizarea Bugului de Sud.

INTRODUCTION

The Southern Bug River is the largest in the West Ukrainian grassland region, with its catchment basin in the densely populated agricultural areas traversed by its numerous tributaries with sources of anthropogenic pollution.

The significant role of phytoplankton for aquatic ecosystems has already been studied (Abacumov, 1978, 1979) and data of its bioindication role in water bodies have also been revealed (Dokulil, 2003; Fedorov, 2004; Barinova et al., 2006, 2013). It is also necessary to uncover the particular role of phytoplankton in river ecosystems (Wehr and Descy, 1998). However, its spatio-temporal variability is relevant mostly to large rivers, which hasn’t been sufficiently examined in the Ukraine. Seasonal observations are not enough; moreover, it isn’t possible to conduct an appropriate ecological assessment and balance estimates to understand the processes that occur under climatic seasonality.

Southern Bug River phytoplankton, in spite of considerable and also modern works carried out (Klochenko and Mytkivska, 1994; Klochenko et al., 1993; Taraschuk, 2004), has not been properly explored in its upper reaches (Bilous et al., 2011). Therefore, it’s necessary to draw attention to phytoplankton distribution and heterogeneity due to seasonal changes.

Our specific studies are more consistently related to bio-indication of the river ecological status, pollution impacts, and self-purification capacities, assessed according to the river management directives (***, 2000; Bajkiewicz-Grabowska, 2011) that emphasize the main importance of phytoplankton characteristics as the most informative component of the large river’s ecosystem. Phytoplankton is obviously responsive to excessive input of inorganic nutrients, posing ecological problems for long stretches of the river affected by eutrophication, usually from land agricultural and industrial sources (Wehr and Descy, 1998).

We started our studies from the upper reaches (Bilous et al., 2011) and have presently extended to investigate the dynamics of phytoplankton communities in the referenced monitoring station at Khmelnitsky to determine the effects of seasonal variation and spatio-temporal variability in the river upper reaches.

MATERIAL AND METHODS

Study Area

The Southern Bug River is one of five large fluvial systems of the Western Steppe region. Out of all Ukrainian rivers, the Southern Bug is the largest river, whose basin belongs only to Ukrainian national territory (Fig. 1). Its catchment encompasses areas of the Volyn-Podolsk plateau and the Black Sea coastal basin (Sukhodolov et al., 2009). The catchment area of the Southern Bug River is approximately 63,700 km^2, with a river length of 806 km (Vyshnevsky, 2000).

The basin territory stretches from the north-west to south-east and is defined by differences in temperature distribution. Thus, for the South Bug basin, wintertime is characterized by precipitation in rain and snow and is sometimes (cold winters) frozen over. The rise in air temperature is accompanied by cloudiness, breeze reduction, foggy days, and frequent thunderstorms in the spring. Clear days with significant temperature increases as well as a rise in precipitation and active thunderstorm activity are common in summer. In turn, autumn is characterized by an increase in cloudy days, continuous precipitation, and long-continuous fogs (Lipinsky et al., 2003).

We chose the Khmelnitsky site as referenced in our studies because it is located in the middle part of the Southern Bug River upper reaches and represents typical situations for the study region.

Figure 1: Map of the study site in the Southern Bug River.

The experiment design

The referenced monitoring station at Khmelnitsky, along the river, was sampled between April 2010 and March 2011, and we also measured hydrochemical variables for ammonia, nitrites, nitrates, and phosphates in summer.

Algological and hydrobiological samples of surface water were collected monthly with Ruttner's bathometer (Romanenko, 2006). The algological samples were investigated using Zeiss and PZO microscopes with living samples as well as fixed samples in a 4% final formaldehyde solution. For quantitative analyses, we used preliminary averaged samples and the counting of cells was carried out in a Nageotte Chamber (0.2 cm^3). In turn, during the counting process, every colony and threadlike organism was considered to be an individual unit. In addition, for accurate definition of the Bacillariophyta species, we prepared permanent slides according to the method of Round (1953).

Taxonomic identification

For taxonomic identification of the potamoplankton taxa, a series of handbooks (Kondrat'eva, 1968; Asaul, 1975; Komárek and Fott, 1983; Starmach, 1983, 1985; Tsarenko, 1990; Krammer and Lange-Bertalot, 1991, 1997a, b, c; Lenzenweger, 1996, 1997, 1999; Krammer, 2000, 2002, 2003; Lange-Bertalot, 2001; Palamar-Mordvinceva, 2003, 2005; Komárek and Anagnostidis, 1998, 2005; Popovský and Pfiester, 2008; Kovalenko, 2009; Levkov, 2009) and selected papers were used (Tsarenko et al., 2005).

Bio-indication

Our ecological analysis has revealed a grouping of freshwater algae indicators to pH, salinity, and saprobity as well as for other habitat conditions (Barinova et al., 2006). Each group was separately assessed with respect to its bioindication significance. Those species that respond to environmental variables can be used as bioindicators reflecting the responses of aquatic ecosystems to eutrophication, pH levels (acidifications), salinity, and organic pollution.

Density-Diversity indices and statistics

Saprobity indices were calculated on the basis of identified species abundance and individual indices:

$$S = \sum_{i=1}^{n}(s_i . a_i) \Big/ \sum_{i=1}^{n}(a_i) \quad (1)$$

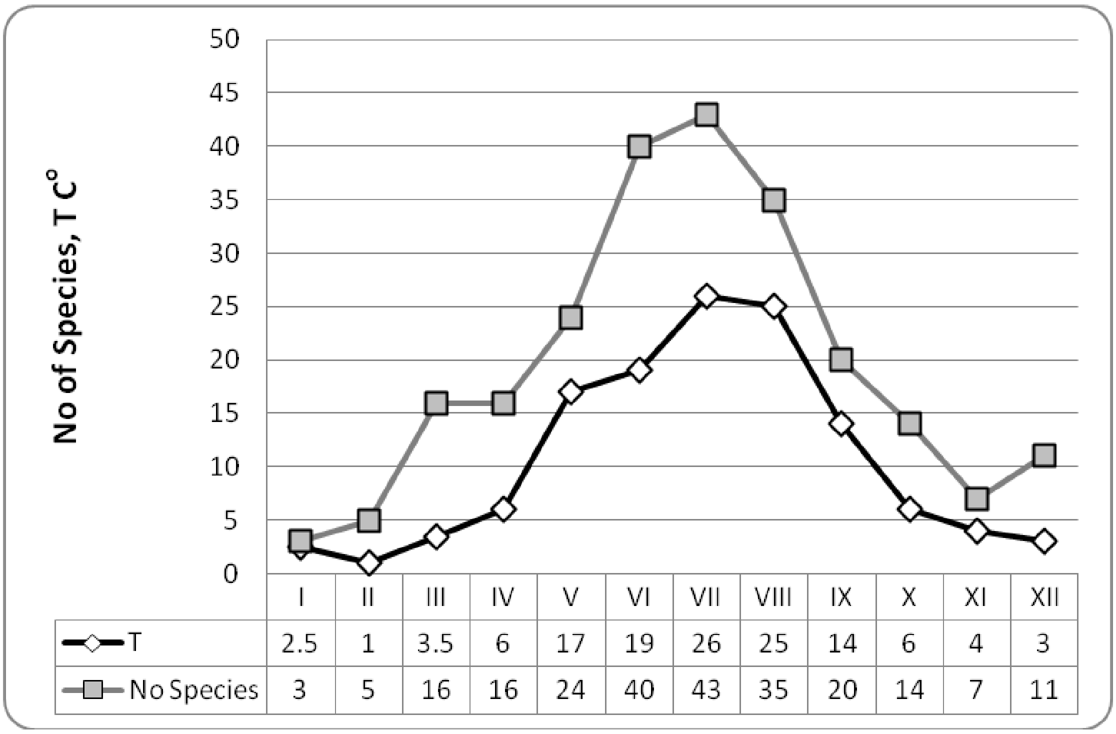
where: S - Index saprobity of algal community; s_i - species-specific saprobity index; a_i - species abundance.

Shannon's diversity index (Odum, 1969) was calculated as:

$$\bar{H} = -\sum_{i=1}^{s} \frac{n_i}{N} \log_2 \frac{n_i}{N} \quad (2)$$

where: N = common organisms abundance, l; s = species number; n_i = species number of every species; = Shannon diversity index, bit.

Statistical methods were used in comparative floristic approaches (Novakovsky, 2004) for calculating similarity of algal communities monthly.



	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
◇ T	2.5	1	3.5	6	17	19	26	25	14	6	4	3
▢ No Species	3	5	16	16	24	40	43	35	20	14	7	11

Figure 2: Water temperature and phytoplankton species richness variation during the year.

As a whole, Chlorophyta species are the richest taxonomic group with a total of 46 taxa, followed by Bacillariophyta (20 taxa), Euglenophyta (13 taxa), Cyanoprokaryota (eight taxa), Dinophyta and Chrysophyta (four taxa each), Streptophyta (two taxa), and Xanthophyta (one taxa). Seasonal dynamics of species distribution in taxonomic divisions during the study period show the complexity of communities in potamoplankton on the Southern Bug River (Fig. 2).

As it can be seen, the role of Bacillariophyta in communities was high in January-March, and diatoms were replaced by green algae during March-September. Blue-green algae developed in summer communities only. It is interesting that the third richest group was euglenoids, which enrich planktonic communities in February-December.

Chlorophyta species were the majority in planktonic communities of the Khmelnitsky site in the Southern Bug River and increased during the vegetation season from March to September with fluctuation till December (Fig. 3). The domination of the Chlorophyta in plankton is quite common for Ukrainian rivers (Vasenko et al., 2002; Vladymyrova, 1976) as well as in the other large rivers of the Black Sea basin (Azari et al., 2011; Marvan et al., 2004). The presence of this division has some variations throughout the study period, as we can see in figure 3, in winter and early spring communities were enriched by Bacillariophyta. The presence of other divisions during the year wasn't so impressive based on the number of species.

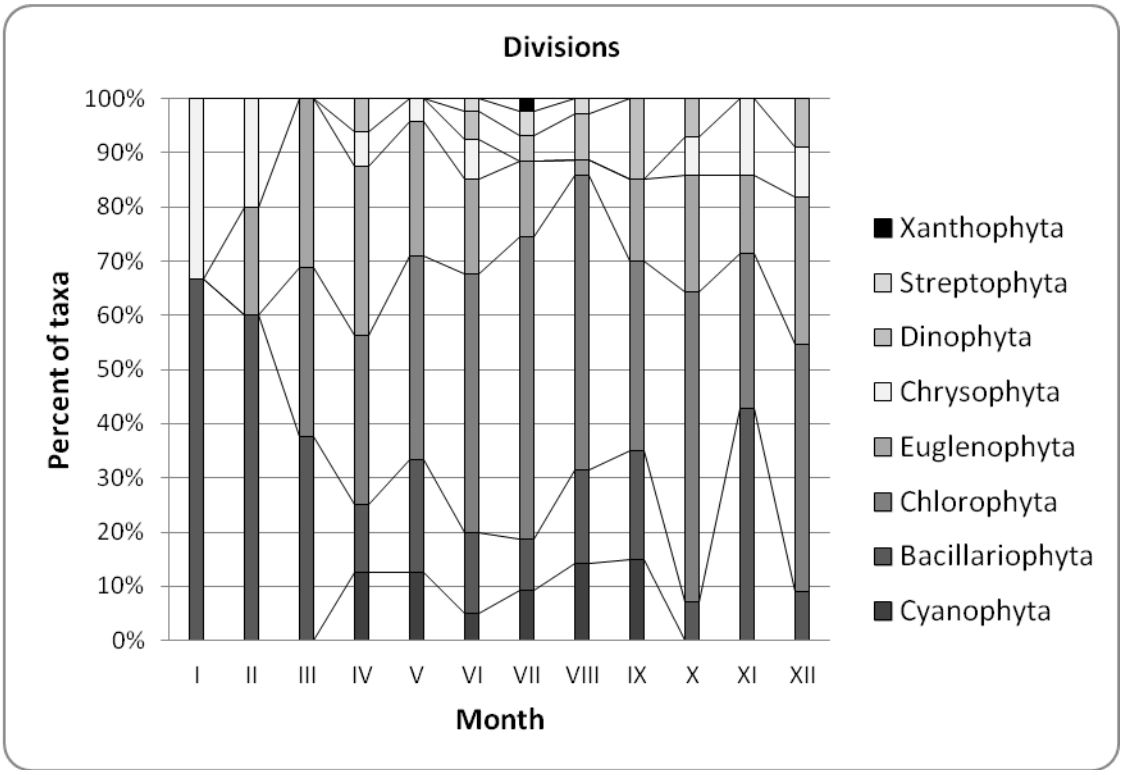


Figure 3: Seasonal dynamics of algal diversity in planktonic communities of the Khmelnitsky site in the Southern Bug River.

Species richness variation represents one peak during the year as seen in figure 2. Therefore, it is difficult to delimit seasonal complexes of phytoplankton. We used a statistical approach to define relationships between planktonic communities' species richness of the Khmelnitsky site (Fig. 4) and revealed seasonal groups of phytoplankton. A comparison of species content overlapping between monthly measured algal diversity shows high similarity between January-February, April-May, and July-August communities.

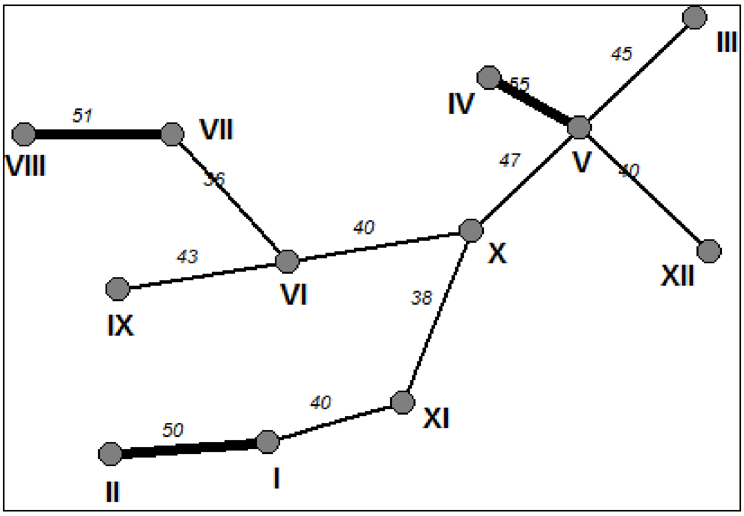


Figure 4: Dendrite of species richness overlapping planktonic communities
in the Khmelnitsky site of the Southern Bug River based on Serensen-Chekanovsky indices;
circles numbered with respect to the sampling month,
bold lines mark relationships between communities.

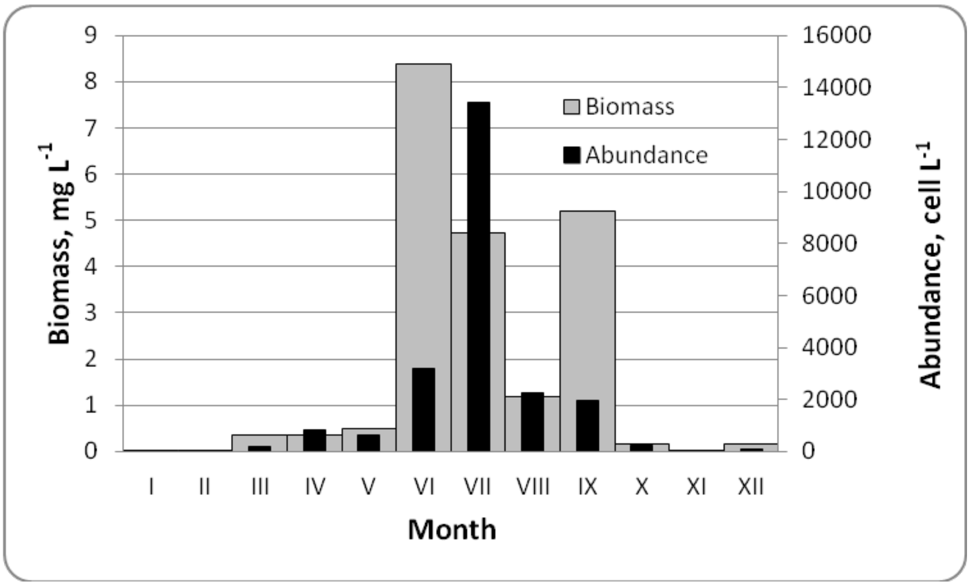


Figure 5: Monthly dynamic of phytoplankton abundance and biomass
in the Khmelnitsky monitoring station
of the southern Bug River.

Algal species abundance and their occurrence by month are represented in Appendix 1. Abundance and biovolume of phytoplankton were maximal in summer with 13.416 mln cells l^{-1} in July and 8.374 mg l^{-1} in June. It's seen that abundance maximum is caused by *Dolichospermum flos-aquae* (Lyngb.) Wacklin, Hoffmann and Komarek with 3.780 mln cells l^{-1}, and biovolume maximum by *Ceratium hirundinella* (O. Müll.) Bergh. with 2.355 mg l^{-1}. In summer we observed the Cyanoprokaryota complex with *Dolichospermum flos-aquae* and *Aphanizomenon flos-aquae* domination. The Dinophyta complex was observed in autumn with the domination of *Ceratium hirundinella*. The Chrysophyta-Bacillariophyta complex was observed in winter, with *Aulacoseira granulata* and *Pseudokephyrion cylindricum* domination. Bacillariophyta complex was observed in the spring, with the domination of *Aulacoseira granulata* and *Melosira varians*.

The phytoplankton abundance and biomass altered with respect to the change in water temperature and can be observed in three seasons during the year (Fig. 5). Remarkably, in southern rivers (which have higher water temperatures and different seasonal aspects), there are only two seasons (Barinova and Tavassi, 2009). The plankton cells' abundance changes are dramatically sharp during the year. Abundance is at a minimum in winter as well as during flooding due to water dilution from melted snow. Plankton abundance and biomass increase in spring with fluctuations related to water level changes. When the level of abundance and biomass is low, water from the plankton rich tributaries, enters the river channel and potamoplankton become more abundant. After summer, the maximum plankton abundance begins to decline due to many organisms transitions at bottom resting forms existence. The number of plankton, leading active lives during the year was not numerous in autumn due to the deterioration of food, and, as a result their rate of reproduction, was decreased (Konstantinov, 1986) as seen in the studied site. Pertaining to the division of distribution, we observed significant abundance and biomass of phytoplankton in the monitoring station for every month of a year. In summer, the most demonstrative division, according to abundance was Cyanoprokaryota; at the same time, greater biomass was observed for the Dinophyta division. This phenomenon easily explains the proper time for vegetation of these groups in this period. Along with lower temperatures, the food quantity decreased causing a decrease in phytoplankton abundance. In autumn Bacillariophyta was noted for its abundance and Dinophyta for its biomass. Distinguished divisions for abundance was Chlorophyta and for biomass – Bacillariophyta in winter time. The cyanobacteria abundance has its major role in the spring, whereas diatoms also formed significant biomass in the spring. We observed strong correlation between abundance and water temperature over the studied year, though some authors (Atici and Obali, 2010) indicated a delay in abundance in comparison with temperature trend. Other chemical variables that we measured in this important river ecosystem study period reflect low-alkaline middle-polluted water, and only ammonia in summer was slightly increased.

To reveal the community complexity fluctuation over climatic seasons, we calculated the Shannon diversity indices on the basis of Appendix 1. As can be seen in figure 6, Shannon index values fluctuated over the year, but as a whole, high values during the summer algal activity can be seen from May till September. Calculated cell biovolume for the planktonic algae community for each month (based on Appendix 1, as a result of dividing the measured monthly biomass of plankton by its cells' abundance) has similar fluctuation with Shannon index all year-round, and it was opposite only in September (Fig. 6).

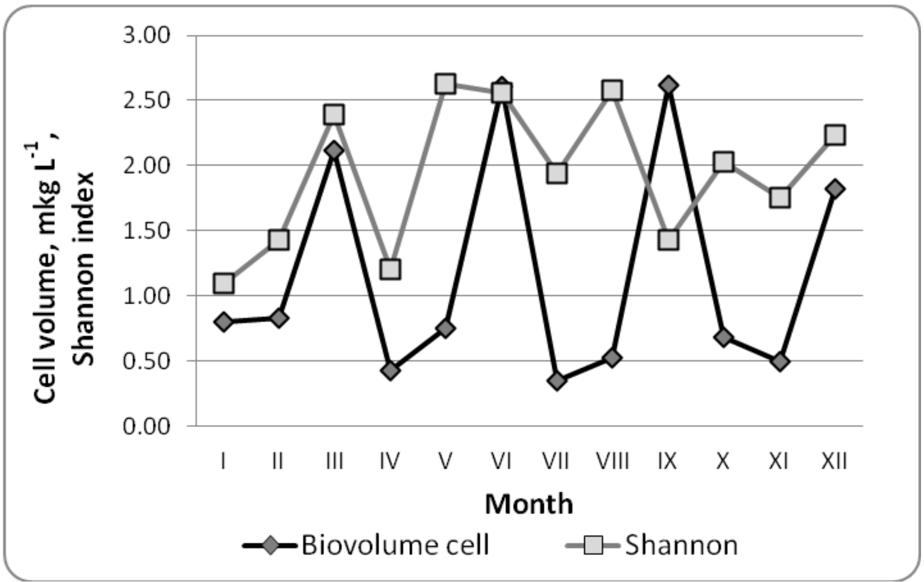


Figure 6: Dynamic of Shannon index and cell biovolume (mkg l^{-1}) calculated for the planktonic communities of the Khmelnitsky site in the southern Bug River.

Figure 6 reveals community complexity correlated with cell biovolume – calculated on the basis of biomass and abundance data (Appendix 1). Periods of decreased cell biovolume reflected small cell species development in communities and therefore community structure change (Barinova et al., 2006; Barinova, 2011; Barinova and Nevo, 2012). In other words, a healthy community state is when species richness is high, algal cells having high volume, and community structure is diverse and complex, e.g., in March, July, and December.

The bioindication analysis of algal species' representation over ecological categories shows (Appendix 2) that the planktonic communities (Appendix 1) included planktonic, plankto-benthic, and some benthic inhabitants. In February and March the plankton were enriched by benthic cells, whereas the community was assembled on 90% of authentic planktonic inhabitants in the ice free period. The temperature indicators changed from cool-water to eurythermic and temperate species during the ice free period from April to November. Indicators of water mass mobility reveal low streaming species over the seasons and indicate the river water as moderately enriched by oxygen. Dynamics of acidity indicator species show low alkaline water with two periods of different species domination, March-July with *Melosira varians* and August-September with *Cymbella lanceolata*. Two groups, mesohalobe and oligohalobious-halophilous species, reveal some peaks of salinity influence over the year. It can be observed in April with *Euglena granulata* and in September-December with *Aphanizomenon flos-aquae* and *Oscillatoria tenuis*.

According to organic pollution, the Class III indicators prevailed during the year. Some groups of algae that are Class V indicators of water quality are also indicators in communities from March till November, dominated by *Melosira varians* and *Fragilaria crotonensis*. Moreover, the number of saprobic species also increased from April-May and September-October. Species ecology data from Appendix 2 show that photosynthetic activity of algal communities described in Van Dam et al. (1994) were very high with mostly autotrophic species over the year, whereas in September, heterotrophic species dominated.

Indicators of a trophic state mostly revealed eutraphentic species during the year. At the same time, we observe an invasion of high trophic state indicators in March, June, and December communities. As a whole, planktonic communities in the referenced monitoring station reflect a medium trophic state of the ecosystem in the studied area of the Southern Bug River.

Bioindication, as a method of environmental variables' biotic assessments, reveals phytoplankton communities' response to environmental changes (Dokulil, 2003). Studied communities on the Southern Bug River site included mostly planktonic and plankto-benthic species which reveals some aspects of river-like reach of the studied part of the river. At the same time we found some benthic forms over a year that indicate water turbulence. As a result of weather conditions and warm temperatures, we can see eurythermic and temperate species increasing from April to November and cool species decreasing at the same time. Moreover, their absence after June, when the temperature is more than 20-25°C, indicated homothermous water in the river.

The water in the monitoring station is moderately oxygenated throughout the year, but from November till January, when mobility of the water mass is less, we recorded an increase in standing water species indicators in communities.

The rise in the salinity level due to melted water, washed away some dissolved solids from the soil's surface. We observed two periods of decreased pH - from March to July and from July till October, which correlate with rainy periods in the Ukraine. Bioindication results also show the impact of salinity during the year where we found two periods of increased water salinity - in April and September-December. It can be correlated also with melting and rainy periods, as we found in water pH fluctuation.

The weather conditions (the snow melting in April) are accompanied with organic pollution, which is confirmed by increases in the alpha-mesosaprobic algal indicators group and can be observed in March-April. The same increase in indicators of pollution can be seen during August-September when the weather is rainy. In the ice free period (March-November) organic pollution is higher than during freezing of the river when the water is protected by snow (as in February). Species indicators, such as saprophilous species, under the Watenabe's organic pollution assessment system, increase abundance in April-May and September-October which indicates an increase in water pollution when the ice cover isn't protected from pollutants and summer species don't consume. Therefore bioindications are revealed flowing from the catchment area during ice melting and rains are the main source of organic pollution.

Due to high abundance and biomass of algae in summer time, especially in July and August, photosynthetic activity of algal communities was higher during the rest of the year. The heterotrophic species indicators, which dominated in September when the abundance and biomass of phytoplankton were lower, correlated with light amount and decrease of water temperature. The increase of eutraphentic species in March, June, and December shows a lower photosynthetic activity level, along with temperature and insolation reduction, which resulted in a decrease in the ecosystem's trophic state. There were a lot of available organics which fluctuated with eutraphentic species and were quite rich in species diversity with abundance and biomass.

We calculated the saprobity indices S on the basis of our database (Barinova et al., 2006) and cell abundance in Appendix 1. The index value fluctuated between 1.73 and 2.32, which indicated Class III water quality during the year. It is remarkable that the highest value of index S was in June and September, but the trend line (Fig. 7) showed highest index values in the summer season.

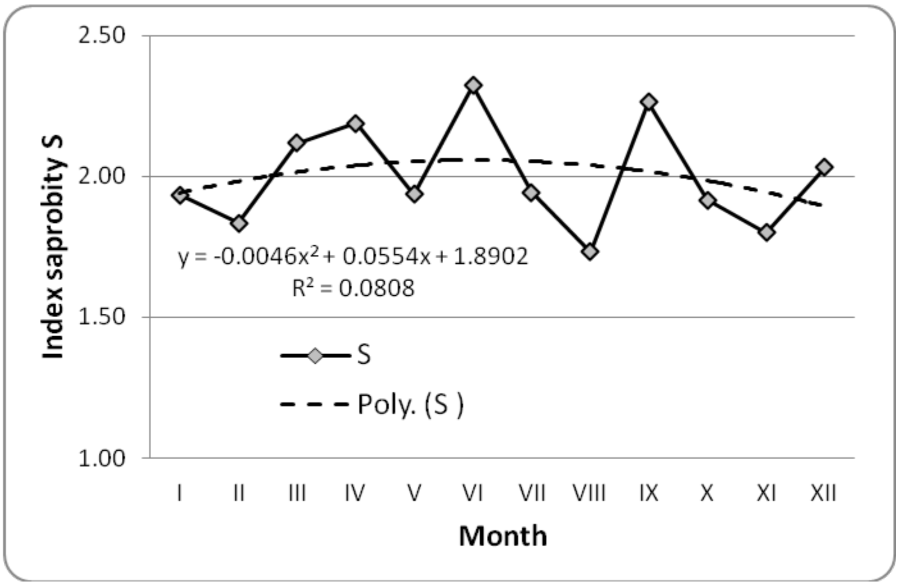


Figure 7: Monthly dynamic of the Saprobity index S during studied period of 2010-2011.

CONCLUSIONS

The planktonic communities of the Khmelnitsky referenced site in the Southern Bug River upper reaches helped us to conclude that river ecosystems have two periods of trophic levels - high in the summer and low in winter. This coincides with abundance and biomass fluctuation and relates mostly with the major environmental variable for the studied river - the water temperature. Bioindication methods, which were implemented for the first time for the southern Bug River helped us characterize the river water in investigating the upper reaches as being low alkaline and low minerals with a moderate organic pollution level. Organic pollution indices fluctuate within the narrow limits, suggesting a relative stability of the river ecosystem that might have coped with organic pollution by adjusting the abundance and biomass of the algal phytoplankton to the seasonal climatic condition.

But the highest value of phytoplankton in June is common in the Black Sea Basin (Solak, 2012). We observed a strong seasonal component in phytoplankton diversity, which is confirmed by the Shannon diversity index. This fact is also shown in other investigations (Şahin et al., 2010; Baykal et al., 2011). Moreover, the abiotic analysis of our investigations confirmed measured biotic parameters as well as the significant role of green algae in planktonic community activity.

As a whole, our analysis shows the important role of temperature in the efficient self-purification ability of the studied river ecosystem. Therefore, climatic seasonality plays a major role in phytoplankton activity in which has the highest activity in summer. In addition, this information can be used in making decisions for the use of water resources for conservation and in the effective utilization of water bodies, such as large rivers in the Ukraine and closely related climatic regions.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Ministry of Absorption of Israel.

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Appendix 1: The phytoplankton abundance (upper: thousand cells l^{-1}) and biomass (bottom: mg l^{-1}) of the Southern Bug River (Khmelnitsky monitoring station) in 2010-2011.

Taxa	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Bacillariophyta												
Achnanthidium minutissima (Kütz.) Czarn.	–	–	4 0.001	5 0.001	30 0.003	–	–	40 0.004	–	–	–	–
Amphora ovalis (Kütz.) Kütz.	–	–	–	–	6 0.015	–	21 0.052	4 0.01	–	–	–	–
Aulacoseira granulata (Ehrenb.) Simonsen var. *granulata*	–	7 0.008	12 0.014	–	–	903 1.083	35 0.042	–	1312 1.574	–	–	–
Cocconeis placentula Ehrenb.	–	–	4 0.008	–	–	–	–	12 0.026	–	–	–	–
Cyclostephanos dubius (Fricke) Round	5 0.005	3.5 0.003	–	–	–	–	–	–	–	–	–	15 0.016
Cyclostephanos invisitatus (Hohn and Hellermann) Theriot. Stoermer and Håkasson	–	–	–	–	–	–	–	–	–	3 0.003	–	–
Cyclotella meneghiniana Kütz.	–	–	–	–	6 0.006	–	–	–	4 0.004	–	–	–
Cymbella lanceolata (C. Agardh) Ehrenb.	–	–	–	–	–	70 0.553	–	–	–	–	–	–
Diatoma mesodon (Ehrenb.) Kütz.	–	3.5 0.004	–	–	–	–	–	–	–	–	–	–
Encyonema caespitosum Kütz.	–	–	–	–	–	–	7 0.012	–	–	–	–	–
Encyonema minuta (Hilse ex Rabenh.) D. G. Mann	–	–	–	–	–	7 0.005	–	–	–	–	–	–
Fragilaria crotonensis Kitton	–	–	–	–	–	–	28 0.002	16 0.002	–	–	–	–
Gomphonema clavatum Ehrenb.	–	–	–	–	–	–	–	–	–	–	4 0.001	–

Appendix 1 (continuing): The phytoplankton abundance (upper: thousand cells l^{-1}) and biomass (bottom: mg l^{-1}) of the Southern Bug River (Khmelnitsky monitoring station) in 2010-2011.

Taxa	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Gomphonema minutum (C. Agardh) C. Agardh	–	–	–	5 0.002	–	–	–	4 0.001	–	–	–	–
Melosira varians C. Agardh	–	–	20 0.132	–	–	826 5.451	–	–	32 0.211	–	–	–
Navicula cryptotenella Lange-Bert.	–	–	16 0.006	–	–	21 0.006	–	–	–	–	4 0.001	–
Navicula tripunctata (O. F. Müll.) Bory	–	–	–	–	6 0.003	–	–	–	–	–	–	–
Stephanodiscus hantzschii Grunow	5 0.006	–	–	–	–	14 0.016	–	–	–	–	4 0.004	–
Ulnaria acus (Kütz.) Aboal	–	–	–	–	–	–	–	16 0.009	4 0.002	–	–	–
Ulnaria ulna (Nitzsch) Compere	–	–	8 0.04	–	48 0.244	–	–	–	–	–	–	–
Chlorophyta												
Actinastrum hantzschii Lagerh. var. *hantzschii*	–	–	–	–	–	–	–	64 0.019	–	–	–	–
Actinastrum hantzschii var. *subtile* Wołosz.	–	–	–	–	–	–	56 0.016	–	–	–	–	–
Acutodesmus obliquus (Turpin) P. Tsarenko	–	–	–	–	–	–	–	32 0.013	–	–	–	–
Acutodesmus pectinatus (Meyen) P. Tsarenko var. *pectinatus*	–	–	–	–	–	35 0.021	28 0.016	16 0.009	–	–	–	–
Coelastrum astroideum De Not.	–	–	–	–	–	140 0.14	336 0.033	132 0.132	36 0.036	–	–	–
Coelastrum microporum Nägeli	–	–	–	–	–	–	154 0.041	72 0.019	–	–	–	–

Appendix 1 (continuing): The phytoplankton abundance (upper: thousand cells l^{-1}) and biomass (bottom: mg l^{-1}) of the Southern Bug River (Khmelnitsky monitoring station) in 2010-2011.

Taxa	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Coenococcus polycoccus (Korschikov) Hindák	–	–	–	–	–	84 0.176	–	–	–	–	–	–
Crucigenia tetrapedia (Kirchn.) West and G. S. West	–	–	–	–	18 0.001	98 0.001	35 0.001	32 0.001	40 0.001	24 0.001	4 0.001	–
Crucigeniella apiculata (Lemmerm.) Komárek	–	–	–	–	–	–	504 0.151	208 0.062	60 0.018	–	–	–
Desmodesmus armatus (Chodat) E. Hegew.	–	–	4 0.002	–	12 0.008	56 0.009	28 0.004	–	–	–	–	–
Desmodesmus bicaudatus (Dedus.) P. Tsarenko.	–	–	–	–	–	–	70 0.009	16 0.002	–	–	–	–
Desmodesmus brasiliensis (Bohlin) E. Hegew.	–	–	–	–	–	–	56 0.022	–	–	–	–	–
Desmodesmus communis (E. Hegew.) E. Hegew. var. *communis*	–	–	–	20 0.005	24 0.006	28 0.007	28 0.007	32 0.008	16 0.004	6 0.001	–	–
Desmodesmus costato-granulatus (Skuja) E. Hegew.	–	–	–	10 0.003	12 0.003	42 0.012	–	–	–	6 0.001	–	–
Desmodesmus denticulatus (Lagerh.) An. Friedl and E. Hegew.	–	–	–	–	–	84 0.016	28 0.005	–	–	–	–	–
Desmodesmus intermedius (Chodat) E. Hegew. var. *acutispinus* (Y. V. Roll) E. Hegew.	–	–	–	–	–	28 0.001	–	–	–	–	–	–

Appendix 1 (continuing): The phytoplankton abundance (upper: thousand cells l^{-1}) and biomass (bottom: mg l^{-1}) of the Southern Bug River (Khmelnitsky monitoring station) in 2010-2011.

Taxa	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Desmodesmus lefevrei (Deflandre) A. Fridl and E. Hege.	–	–	–	–	–	28 0.008	–	–	–	–	–	–
Desmodesmus subspicatus (Chodat) E. Hegew. and A. Schmidt var. *subspicatus*	–	–	–	–	24 0.007	–	–	–	–	–	–	–
Dictyosphaerium granulatum Hindák	–	–	–	–	–	–	56 0.002	–	–	–	–	–
Dictyosphaerium pulchellum Wood	–	–	–	–	–	84 0.004	–	–	–	–	–	–
Enallax acutiformis (Schröd.) Hindák var. *acutiformis*	–	–	–	–	–	–	–	–	16 0.009	–	–	–
Enallax acutiformis (Schröd.) Hindák var. *costatus* (Hub.-Pest.) P. Tsarenko	–	–	–	–	–	–	28 0.008	–	–	–	–	–
Granulocystopsis coronata (Lemmerm.) Hindák	–	–	–	–	–	–	7 0.001	–	–	–	–	–
Kirchneriella lunaris (Kirchn.) Moeb.	–	–	–	–	–	14 0.001	7 0.001	–	–	3 0.001	4 0.001	–
Koliella longiseta (Vischer) Hindák	–	–	–	–	–	7 0.001	–	–	–	–	–	15 0.001
Lagerheimia wratislaviensis Schröd.	–	–	–	–	–	–	7 0.001	–	–	–	–	–
Micractinium pusillum Fresen.	–	–	–	–	–	–	–	–	–	–	–	20 0.001
Monactinus simplex (Meyen) Corda	–	–	–	–	–	–	–	4 0.002	–	–	–	–

Appendix 1 (continuing): The phytoplankton abundance (upper: thousand cells l^{-1}) and biomass (bottom: mg l^{-1}) of the Southern Bug River (Khmelnitsky monitoring station) in 2010-2011.

Taxa	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Monactinus simplex (Meyen) Corda var. *echinulatum* (Wittr.) P. Tsarenko	–	–	–	–	–	–	–	32 0.019	–	–	–	–
Monoraphidium arcuatum (Korschikov) Hindák	–	–	12 0.002	–	12 0.002	7 0.001	–	–	4 0.001	6 0.001	–	5 0.001
Monoraphidium griffithii (Berk.) Komárk.-Legn.	–	–	8 0.001	10 0.001	6 0.001	35 0.003	70 0.007	12 0.001	–	6 0.001	–	5 0.001
Monoraphidium minutum (Nägeli) Komárk.-Legn.	–	–	8 0.001	5 0.001	6 0.001	–	21 0.001	4 0.001	–	–	–	5 0.001
Oocystis lacustris Chodat	–	–	–	–	–	–	49 0.029	–	–	–	–	–
Pediastrum duplex Meyen var. *duplex*	–	–	–	–	–	–	98 0.029	64 0.02	–	–	–	–
Pediastrum duplex var. *subgranulatum* Racib.	–	–	–	–	–	–	98 0.029	–	–	–	–	–
Pseudopediastrum boryanum (Turpin) E. Hegew.	–	–	–	–	–	–	–	60 0.012	–	–	–	–
Pseudopediastrum boryanum var. *longicorne* (Reinsch) P. Tsarenko	–	–	–	–	–	–	49 0.024	–	–	–	–	–
Raphidocelis sigmoidea Hindak	–	–	–	5 0.001	–	–	–	–	–	–	–	–
Scenedesmus ellipticus Corda	–	–	–	–	24 0.009	–	–	16 0.006	–	–	–	–
Scenedesmus obtusus Meyen var. *obtusus*	–	–	–	–	–	28 0.011	–	–	–	–	–	–

Appendix 1 (continuing): The phytoplankton abundance (upper: thousand cells l^{-1}) and biomass (bottom: mg l^{-1}) of the Southern Bug River (Khmelnitsky monitoring station) in 2010-2011.

Taxa	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Tetraedron minimum (A. Braun) Hansg.	–	–	–	–	–	63 0.018	70 0.021	12 0.004	4 0.001	–	–	–
Tetraedron triangulare Korschikov	–	–	–	–	–	28 0.014	–	–	–	–	–	–
Tetrastrum staurogeniaeforme (Schröd.) Lemmerm.	–	–	–	–	–	–	–	–	–	12 0.001	–	–
Tetrastrum triangulare (Chodat) Komárek	–	–	48 0.024	–	–	112 0.056	–	64 0.032	–	48 0.024	–	–
Treubaria triappendiculata C. Bernard	–	–	–	–	–	–	–	4 0.001	–	–	–	–
Westella botryoides (W. West) De Wild.	–	–	–	–	–	–	28 0.005	–	–	–	–	–
Chrysophyta												
Dinobryon divergens Imhof	–	–	–	–	–	7 0.025	–	–	–	–	–	–
Pseudokephyrion cylindricum (Lackey) Bourr.	5 0.001	14 0.004	–	35 0.01	30 0.009	–	–	–	–	78 0.023	16 0.004	10 0.003
Pseudokephyrion latum (J. Schiller) W. G. G. Schmid	–	–	–	–	–	7 0.002	–	–	–	–	–	–
Pseudokephyrion schilleri (J. Schiller) W. Conrad	–	–	–	–	–	7 0.002	–	–	–	–	–	–
Cyanoprokaryota												
Aphanizomenon elenkinii Kisselev	–	–	–	–	–	–	3714 0.296	76 0.006	–	–	–	–
Aphanizomenon flos-aquae (L.) Ralfs ex Bornet and Flahault	–	–	–	600 0.048	–	–	3388 0.271	136 0.011	–	–	–	–

Appendix 1 (continuing): The phytoplankton abundance (upper: thousand cells l^{-1}) and biomass (bottom: mg l^{-1}) of the Southern Bug River (Khmelnitsky monitoring station) in 2010-2011.

Taxa	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Aphanocapsa planctonica (G. M. S.) Komárek and Anagn.	–	–	–	–	–	105 0.002	–	–	–	–	–	–
Dolichospermum flos-aquae (Lyngb.) Wacklin. Hoffmann and Komarek	–	–	–	–	–	112 0.008	3780 0.302	52 0.004	84 0.006	–	–	–
Microcystis aeruginosa (Kütz.) Kütz.	–	–	–	–	90 0.005	–	280 0.016	–	–	–	–	–
Microcystis firma (Bréb. and Lenor.) Schmidle	–	–	–	–	–	–	–	772 0.015	–	–	–	–
Oscillatoria amphibia J. Agardh ex Gomont	–	–	–	–	168 0.016	–	–	196 0.002	80 0.001	–	–	–
Oscillatoria tenuis Agardh ex Gomont	–	–	–	50 0.005	60 0.006	–	–	–	160 0.016	–	–	–
Dinophyta												
Ceratium hirundinella (O. Müll.) Bergh	–	–	–	–	–	7 0.214	77 2.355	12 0.367	104 3.182	–	–	–
Gymnodinium paradoxum A. J. Schill	–	–	–	10 0.136	–	–	–	4 0.054	4 0.054	–	–	–
Peridiniopsis polonicum (Wołosz.) Bourr.	–	–	–	–	–	21 0.277	–	–	4 0.052	3 0.039	–	–
Peridinium aciculiferum Lemmerm.	–	–	–	–	–	–	7 0.17	12 0.293	–	–	–	5 0.122
Euglenophyta												
Euglena acus Ehrenb.	–	–	–	–	6 0.006	–	7 0.007	–	–	–	–	5 0.005
Euglena granulata (G. A. Klebs) Schmitz	–	–	–	5 0.027	–	–	–	–	–	–	–	–

Appendix 1 (continuing): The phytoplankton abundance (upper: thousand cells l^{-1}) and biomass (bottom: mg l^{-1}) of the Southern Bug River (Khmelnitsky monitoring station) in 2010-2011.

Taxa	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Euglena oblonga Schmitz	–	–	4 0.082	–	–	–	–	–	–	–	–	–
Lepocinclis fusiformis (Carter) Lemmerm.	–	–	–	–	–	–	14 0.096	–	–	–	–	–
Phacus acuminatus A. Stokes	–	–	–	–	–	14 0.03	–	–	–	–	–	–
Phacus curvicauda Svirenko	–	–	4 0.015	–	–	–	7 0.026	–	–	–	–	–
Phacus longicauda (Ehrenb.) Dujard. var. *longicauda* f. *longicauda*	–	–	–	–	–	7 0.019	14 0.039	4 0.011	–	–	–	–
Phacus orbicularis Hübner	–	–	–	–	–	–	21 0.254	–	–	–	–	–
Trachelomonas nigra Swir.	–	–	–	10 0.036	12 0.043	7 0.025	–	–	–	–	–	5 0.018
Trachelomonas volvocina Ehrenb. var. *coronata* Lemmerm.	–	–	–	5 0.01	12 0.025	7 0.014	–	–	4 0.008	3 0.006	–	–
Trachelomonas volvocina Ehrenb. var. *volvocina*	–	3.5 0.007	4 0.008	–	24 0.05	28 0.058	63 0.132	–	8 0.016	15 0.031	–	–
Trachelomonas volvocina var. *punctata* Playfair	–	–	4 0.008	25 0.052	6 0.012	21 0.044	–	–	–	9 0.018	4 0.008	–
Trachelomonas volvocinopsis Svirenko	–	–	4 0.003	15 0.012	6 0.004	14 0.011	–	–	8 0.006	–	–	5 0.004
Streptophyta												
Cosmarium bioculatum Brébisson ex Ralfs	–	–	–	–	–	7 0.029	14 0.058	8 0.034	–	–	–	–
Cosmarium lapponicum Borge	–	–	–	–	–	–	21 0.132	–	–	–	–	–
Xanthophyta												
Ophiocytium capitatum Wolle	–	–	–	–	–	–	7 0.009	–	–	–	–	–

Appendix 2: The algal indicators in the communities of the Southern Bug River (Khmelnitsky station) with species autecology (Barinova et al., 2006).

Species	Hab	T	Oxy	D	Sal	pH	S	Het	Tro
Bacillariophyta									
Achnanthidium minutissima	–	–	–	–	–	–	–	–	–
Amphora ovalis	B	temp	st-str	sx	i	alf	a-b	ate	e
Aulacoseira granulata granulata	–	–	–	–	–	–	–	–	–
Cocconeis placentula	P-B	temp	st-str	es	i	alf	o-b	ate	e
Cyclostephanos dubius	–	–	st-str	–	hl	alb	o-b	ate	e
Cyclostephanos invisitatus	–	–	–	es	–	–	o-b	–	–
Cyclotella meneghiniana	P-B	temp	st	sp	hl	alf	o-a	hne	e
Cymbella lanceolata	B	–	str	sx	i	alf	o	ats	o-e
Diatoma mesodon	B	cool	st-str	sx	hb	neu	o-b	ats	m
Encyonema caespitosum	B	–	–	sx	–	–	b-a	–	–
Encyonema minuta	–	–	–	–	–	–	–	–	–
Fragilaria crotonensis	P	–	St	es	Hl	alf	a-b	ate	m
Gomphonema clavatum	B	–	str	es	i	ind	o-b	ats	me
Gomphonema minutum	B	–	–	es	oh	alf	o-b	–	e
Melosira varians	P-B	temp	st-str	es	hl	alf	a-b	hne	e
Navicula cryptotenella	B	–	–	sx	i	ind	o-b	–	o-e
Navicula tripunctata	B	–	st-str	es	i	ind	b	ate	e
Stephanodiscus hantzschii	P	temp	st	es	i	alf	a-b	hne	he
Ulnaria acus	P	–	st-str	es	i	alb	o-a	–	–
Ulnaria ulna	B	temp	st-str	es	i	alf	b-o	ate	o-e
Chlorophyta									
Actinastrum hantzschii hantzschii	P-B	–	st-str	–	i	–	b	–	–
Actinastrum hantzschii subtile	P-B	–	–	–	i	–	b	–	–
Acutodesmus obliquus	–	–	–	–	–	–	–	–	–
Acutodesmus pectinatus pect.	–	–	–	–	–	–	–	–	–
Coelastrum astroideum	P	–	st-str	–	–	–	b	–	–
Coelastrum microporum	P-B	–	st-str	–	i	ind	b	–	–
Coenococcus polycoccus	–	–	–	–	–	–	–	–	–
Crucigenia tetrapedia	P-B	–	st-str	–	i	ind	o-a	–	–
Crucigeniella apiculata	P-B	–	st-str	–	–	–	b	–	–
Desmodesmus armatus	P-B	–	st-str	–	–	–	o-a	–	–
Desmodesmus bicaudatus	–	–	–	–	–	–	–	–	–
Desmodesmus brasiliensis	P-B	–	st-str	–	–	–	b	–	–
Desmodesmus communis com.	–	–	–	–	–	–	–	–	–
Desmodesmus costato-granulatus	P-B	–	st-str	–	–	–	b	–	–
Desmodesmus denticulatus	P-B	–	st-str	–	i	–	b	–	–
Desmodesmus intermedius acut.	–	–	–	–	–	–	–	–	–
Desmodesmus lefevrei	–	–	–	–	–	–	b	–	–
Desmodesmus subspicatus subsp.	P-B	–	st-str	–	–	–	o	–	–
Dictyosphaerium granulatum	–	–	–	–	–	–	–	–	–
Dictyosphaerium pulchellum	P-B	–	st-str	–	i	ind	b	–	–

Appendix 2 (continuing): The algal indicators in the communities of the Southern Bug River (Khmelnitsky station) with species autecology (Barinova et al., 2006).

Species	Hab	T	Oxy	D	Sal	pH	S	Het	Tro
Enallax acutiformis acutiformis	–	–	–	–	–	–	–	–	–
Enallax acutiformis var. *costatus*	–	–	–	–	–	–	–	–	–
Granulocystopsis coronata	–	–	–	–	–	–	b	–	–
Kirchneriella lunaris	P-B	–	st-str	–	i	–	b	–	–
Koliella longiseta	P	–	st	–	i	–	b	–	–
Lagerheimia wratislaviensis	P-B	–	st-str	–	–	–	b	–	–
Micractinium pusillum	P-B	–	st-str	–	–	–	b-a	–	–
Monactinus simplex	–	–	–	–	–	–	–	–	–
Monactinus simplex echinulatum	–	–	–	–	–	–	–	–	–
Monoraphidium arcuatum	P-B	–	st-str	–	–	–	b	–	–
Monoraphidium griffithii	P-B	–	st-str	–	–	–	b	–	–
Monoraphidium minutum	P-B	–	st-str	–	–	–	b-a	–	–
Oocystis lacustris	P-B	–	st-str	–	hl	–	b-o	–	–
Pediastrum duplex var. *duplex*	P	–	st-str	–	i	ind	o-a	–	–
Pediastrum duplex subgranul.	P-B	–	st-str	–	–	–	–	–	–
Pseudopediastrum boryanum		–	–	–	–	–	–	–	–
Pseudopediastrum boryanum lon.	–	–	–	–	–	–	–	–	–
Raphidocelis sigmoidea	P	–	st-str	–	–	–	–	–	–
Scenedesmus ellipticus	P-B, S	–	st-str	–	–	–	o-b	–	–
Scenedesmus obtusus obtusus	–	–	–	–	–	–	–	–	–
Tetraedron minimum	P-B	–	st-str	–	i	–	b	–	–
Tetraedron triangulare	P-B	–	st-str	–	i	–	b	–	–
Tetrastrum staurogeniaeforme	P-B	–	st-str	–	i	–	b	–	–
Tetrastrum triangulare	P-B	–	st-str	–	–	–	b	–	–
Treubaria triappendiculata	P-B	–	st-str	–	–	–	–	–	–
Westella botryoides	P	–	st-str	–	–	–	b	–	–
Chrysophyta									
Dinobryon divergens	P	–	st-str	–	i	ind	o-a	–	–
Pseudokephyrion cylindricum	–	–	–	–	–	–	b-o	–	–
Pseudokephyrion latum	–	–	–	–	–	–	–	–	–
Pseudokephyrion schilleri	–	–	–	–	–	–	–	–	–
Cyanoprokaryota									
Aphanizomenon elenkinii	B	–	–	–	–	–	b-o	–	–
Aphanizomenon flos-aquae	P	–	–	–	hl	–	b	–	–
Aphanocapsa planctonica	P	–	–	–	i	–	–	–	–
Dolichospermum flos-aquae	–	–	–	–	–	–	–	–	–
Microcystis aeruginosa	P	–	–	–	hl	–	o-a	–	–
Microcystis firma	P	–	–	–	–	–	o	–	–
Oscillatoria amphibia	P-B, S	–	st-str	–	hl	–	o-a	–	–
Oscillatoria tenuis	P-B, S	–	st-str	–	hl	–	b-a	–	–
Dinophyta									
Ceratium hirundinella	P	–	st-str	–	i	–	o	–	–
Gymnodinium paradoxum	–	–	–	–	–	–	–	–	–

Appendix 2 (continuing): The algal indicators in the communities of the Southern Bug River (Khmelnitsky station) with species autecology (Barinova et al., 2006).

Peridiniopsis polonicum	P	–	st	–	–	–	–	–	–
Peridinium aciculiferum	–	–	–	–	–	–	o-b	–	–
Euglenophyta									
Euglena acus	P	eterm	st	–	i	ind	b	–	–
Euglena granulata	P	eterm	st-str	–	mh	ind	b-a	–	–
Euglena oblonga	P	eterm	st-str	–	Ph	ind	b	–	–
Lepocinclis fusiformis	P	eterm	st-str	–	i	ind	b	–	–
Phacus acuminatus	P-B	eterm	st-str	–	i	–	b-a	–	–
Phacus curvicauda	P-B	–	st	–	i	ind	b	–	–
Phacus longicauda longicauda	–	–	–	–	–	–	–	–	–
Phacus orbicularis	P-B	–	st-str	–	i	ind	b	–	–
Trachelomonas nigra	P	cool	st-str	–	hl	–	b	–	–
Trachelomonas volvocina coron.	–	–	–	–	–	–	–	–	–
Trachelomonas volvocina volv.	B	eterm	st-str	–	i	ind	b	–	–
Trachelomonas volvocina punct.	–	–	–	–	–	–	–	–	–
Trachelomonas volvocinopsis	P	–	st-str	–	i	–	b	–	–
Streptophyta									
Cosmarium bioculatum	P-B	–	st-str	–	hb	–	–	–	–
Cosmarium lapponicum	–	–	–	–	–	–	–	–	–
Xanthophyta	–	–	–	–	–	–	–	–	–
Ophiocytium capitatum	P	–	st	–	oh	–	o	–	–

* Ecological types (Hab): B - benthic; P-B - planktic-benthic; S - soil; pb - phycobiont; P - planktonic. Temperature (T): temp - temperate; eterm - eurythermic; cool - cool. Streaming and oxygenation (Oxy): st - standing water; st-str - standing-streaming. Saprobity categories of Watanabe et al. (1986) (D): es - eurysaprob; sx - saprogen; sp-saprophil. Groups of salinity indicators (Husted, 1957) (Sal): mh - mesohalobe; I - oligohalobious-indifferent; hl - oligohalobious-halophilous; hb - oligohalobious-halophobous. Acidity (pH) (Hustedt, 1957): ind - indifferent; alf - alkaliphil; acf - acidophil; alb - alkalibiont. Saprobity (Sládeček, 1986) (Sap): o - oligosaprob; b - beta-mesosaprob; b-o - beta-oligomesosaprob; o-a - oligo-alpha-mesosaprob; b-a - beta-alphamesosaprob; a-b - alphamesosaprob; o-b - oligo-betamesosaprob. Nitrogen uptake metabolism (Het) (Van Dam et al., 1994): ats - nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate - nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; hne - facultative nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen. Trophic state (Tro) (Van Dam et al., 1994): m - mesotraphentic; me - meso-eutraphentic; he - hypereutraphentic; e - eutraphentic; o-e - oligo- to eutraphentic (hypereutraphentic).

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THE ECONOMIC AND ECOLOGICAL POTENTIAL OF MACROPHYTIC VEGETATION IN URBAN LAKES

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DOI: 10.2478/trser-2013-0021

KEYWORDS: Macrophytes, urban lakes, Bucharest, fertiliser, fodder, integrated waste management, integrated lake management.

ABSTRACT

This paper considers certain elements relating to the ecological and economic importance of the aquatic macrophytes common in the Câmpia Română/Romanian Plain lakes, especially those species belonging to the genera *Myriophyllum* and *Potamogeton*, common in Bucharest's urban lakes. At present, the macrophytes of the Bucharest lakes are collected by personnel of the Lakes, Parks and Leisure Administration of Bucharest by mechanised and manual methods, which are sun-dried and transported as waste and deposited in a landfill. Thus these macrophytes are merely generating costs through harvest, transport and storage, instead of being a direct revenue source for the City hall. This study presents a review of international literature in support of the economic potential as fertiliser for open field crop, orchard and garden crop application, as well as food for farm animals. The last portion of the paper argues the vital need for the preservation of macrophyte stands in the lacustrine habitat for green, sustainable and integrated management of the urban lakes used for leisure and fishing.

RÉSUMÉ: Le potentiel économique et écologique de la végétation macrophytique provenant des lacs urbains.

L'article présente les aspects de l'importance économique et écologique des espèces de plantes macrophytes communes, dans les lacs de la plaines roumaine, et particulièrement les espèces *Myriophyllum* et *Potamogeton* peuplant les lacs urbains de Bucarest. A présent, les macrophytes de ces lacs sont ramassés par le personnel de l'Administration des Lacs, Parcs et Loisirs de Bucarest par des moyens mécanisés ou à la main, séchés au soleil puis transportés à la déchèterie municipale. Ainsi les plantes génèrent uniquement des coûts de cueillette, transport et stockage, sans apporter de bénéfice économique direct. L'article recence quelques publications scientifiques internationales documentant l'usage des macrophytes comme engrais et protection pour les cultures, les vergers et les jardins ainsi qu'en tant que supplément nutritif pour les animaux de ferme. Ensuite, on présente la nécessité vitale de préserver quelques lopins de macrophytes dans les habitats lacustres afin d'assurer une gestion durable, écologique et intégrée des lacs utilisés pour les loisirs et la pêche.

REZUMAT: Potenţialul economic şi ecologic al vegetaţiei macrofitice din lacurile urbane.

Prezenta lucrare abordează elemente legate de importanţa ecologică şi economică a speciilor de plante macrofite acvatice comune lacurilor din Câmpia Română, în special a speciilor din genul *Myriophyllum* şi *Potamogeton*, în cazul lacurilor urbane bucureştene. În prezent, macrofitele din lacurile din Bucureşti sunt recoltate de personalul Administraţiei Lacuri, Parcuri şi Agrement Bucureşti cu mijloace mecanizate sau manuale, deshidratate natural şi transportate la groapa de gunoi municipală ca deşeuri. Acest lucru face ca plantele să antreneze doar costuri substanţiale, de recoltare, de transport şi de depozitare, fără să aducă nici un beneficiu economic direct. Lucrarea constituie o trecere în revistă a publicațiilor științifice internaționale ce tratează utilizarea macrofitelor ca îngrăşământ şi protector de culturi de câmp, livadă şi grădină precum şi ca hrană pentru animale. Lucrarea tratează de asemenea necesitatea vitală a conservării unor parcele populate cu aceste plante în habitatul lacustru pentru o administrare ecologică, durabilă şi integrată a corpurilor de apă utilizate pentru agrement şi piscicultură cu argumente din date şi observaţii proprii.

INTRODUCTION

The most common macrophytes in Bucharest lakes are the milfoil *Myriophyllum spicatum* (Linné, 1753) Magnoliopsida, Saxifragales, Haloragidaceae and the curly-leaf pondweed *Potamogeton crispus* (Linné, 1753), Liliopsida, Najadales, Potamogetonaceae. These species colonise lakes such as Tineretului Lake, Lia Manoliu Lake or National Park Lake, Titan Park Lake, Tei Lake, Herăstrău Lake and the urban portion of the Dâmboviţa River channel. *Potamogeton* is the most commonly found macrophyte. Growing up to 3-4 m long, Potamogeton has many leaves and may cover entire lake surfaces, especially in urban lakes less than 2 m deep. This particular biotope forms a suitable habitat for migrating waterfowl that nest and feed on the leaves (especially the ducklings who consume them in large quantities). Underwater, an entire ecosystem has been established with macrophyte species of phytoplankton, zooplankton, epiphytes, water insects, Orthoptera larvae, arachnids, and predator coleopterans. There are also alevins of different fish species, in particular economically and ecologically interesting predators such as pike or perch, and the presence of turtles (*Emys orbicularis*, a CITES Convention protected and Romania’s Red Book mentioned species, counts at least a dozen individuals in Tineretului Lake for instance) and amphibians (some of the species being protected by the law applying the CITES Convention). The milfoil is particularly important since it is used as a nursery by several freshwater fish species. From personal observations, I have observed that the plant has quite an important capacity to propagate with cuttings, unlike the pondweed which does not survive without a consequent substratum and without intact roots.

Presently, macrophytes are considered by the public to be an invasive species, aesthetically displeasing, or at least useless plants. Thus, under the pressure of the public, they are harvested and treated as urban waste with the Environmental Guard to the lake, and Administration issuing fines when macrophytes are not harvested and deposed of properly. Such practices are not recommended by biologists, biochemists and even agricultural engineers. Their arguments are presented below. The areas colonised by aquatic macrophytes could be put to use according to integrated management techniques gaining economic benefits from the sale of macrophytes to agricultural users. There are also economic benefits from the sustainable and integrated management of the lake ecosystems. By conserving patches of macrophytes for repopulation and for the protection of alevins and hatchlings, as many aquatic birds nest on urban lakes. Their economical potential is capitalised by using the plants as fertilisers and crop protection, as well as a nutritional supplement for farm animals. Last but not least, the ecological importance of aquatic macrophytes is generally known and was demonstrated also in the case of the Tineretului Lake of Bucharest. The macrophytes influence the physical and chemical parameters of the lake water, as well as contribute to the local micro and macrofauna. The lake is used for fishing, populated with perch, pike, carp, Prussian carp, bighead carp, silver carp, catfish and common rudd. Significant mallard and herring gull populations use the lake as a nesting site along with families of little grebe and common moorhen, which are attracted by the presence of the submerse vegetation.

MATERIAL AND METHODS

For the present paper the documentation elaborated by the international authority in the field, the Food and Agriculture Organisation (FAO), as well as international studies regarding the ecology of the two macrophyte species are reviewed.

Own observations and communications with personnel administering the Tineretului Lake were also used to evaluate the impact of the macrophyte presence on the macrofauna.

Samples of zooplankton and alevins were collected during 2007 and 2009 from Tineretului Lake. Also, physical and chemical readings such as water and air temperature, transparency, dissolved oxygen and carbon dioxide, bicarbonate, nitrate, nitrite, phosphate ions and ammonia were measured in Tineretului Lake during the same period.

RESULTS

Economical advantages of using aquatic macrophytes

Reimer and Toth (1969), performed detailed chemical analysis on several species of *Potamogeton* and *Myriophyllum*, as well as on species of Nymphaeaceae, in order to determine the nutrient uptake potential of every species. Ample variations of the chemical composition were registered, both intra and interspeciphically. It is notable that these samples were taken on plants coming from the same water tank in New Jersey, USA. The averages are presented in table number 1.

The rich content of potassium, iron, phosphorus and trace elements suggests using these plants as fertilisers. Putrefying weeds have historically been used in poor agricultural areas as fertilisers. It is a simple and efficient use for these plants. Aquatic macrophytes harvested and then naturally desiccated on dry land have the advantage that their seeds and roots will not compete with the crop, as is the case with weeds. This source of minerals and organic nutrients is recommended due to low harvesting costs and treatment, especially for areas in which these plants are abundant and artificial fertilisers are rare and expensive (Kamal and Little, 1970).

In horticulture, applying thick layers of last year's aquatic plants on the soil early in the spring (previously dried and partially decomposed) will inhibit the growth of weeds and maintain moisture in the soil. Also, the aquatic plants can be used for mulching. When decomposition is finished, all nutrients are absorbed into the soil and the fibrous remains can be incorporated in the soil as a supplement of organic matter with the added benefit of aerating clay-rich soil.

When cultivated land is far from the water bodies, drying the plants on shore in advance will minimise transportation and operating costs by diminishing weight and volume. Another option is composting the aquatic plants, at the end of this process the product being less voluminous, is easier to transport. Some authors propose burning the dry plants and using the ash as fertiliser, since it contains a higher concentration of minerals, except nitrogen, and it weighs only a fraction of the original wet-plant mass. But in climate conditions that do not allow the complete dehydration of the plants prior to burning this operation can be inconvenient.

Reimer and Toth (1969) stress that, "The analytical data indicate that species of *Myriophyllum* and *Potamogeton* contain sufficient total N for decomposition. Partial drying of the cut plants before composting, however, will be necessary to reduce anaerobic decomposition to a minimum. The final composts prepared from the aquatic plants probably will contain fairly high amounts of minor elements, especially Fe, Mn and Zn, which may be beneficial (Tab. 1). It is believed that the composts will need the addition of extra P and K."

Table 1: Average concentrations of minerals in plants of the *Potamogeton* genera, including *P. amplifolius*, *P. crispus*, *P. natans*, *P. pulcher* and *P. pectinatus*, and the *Myriophyllum* genera, including *M. heterophyllum* and *M. spicatum*, after Reimer and Toth (1969).

Element	*Potamogeton* spp.	*Myriophyllum* spp.
	% dry weight	% dry weight
Na	0.56	1.15
K	2.28	1.75
Ca	3.38	1.38
Mg	0.33	0.32
Fe	0.73	2.10
Mn	0.16	0.76
P	0.33	0.43
S	0.44	0.29
N	2.51	2.81
Cl	1.18	1.86
Cu	33.9	35.0
Zn	82.9	379.0
	ppm	ppm

Thompson and Hartwig (1973) also reported the successful use of *Myriophyllum spicatum* and *M. heterophyllum* as soil fertilisers. Plants were prepared by being cut and dried.

Other uses of these plants exist in places like Canada, the United States, China, India and Egypt, namely as horticulture and greenhouse-crops manure, fertilisers for potted plants, winter fertilisers for orchards and turf, and foliar fertilisers. These practices are employed by individuals, as well as by small-scale producers, with excellent results and are encouraged by receptive eco-oriented markets in Western Europe and North America.

The manufacturing costs for ecological aquatic-plant-based fertilisers are much lower than that of artificial fertilisers. When combined with the large interest in the western market, combined with the significant demand for retail sales of this type of product. It points to a particularly noteworthy profit share for the businesses interested in this market segment. Ecological fertilisers have even generated a fashion trend in Canada called the Lasagna Garden trend. The “algae” powder contains several trace elements in higher concentrations than are found in artificial fertilisers with the main nutrient ratio being N-K-P = (1.5-0.2-1.3). The powder is used to stimulate growth in plants and increase their hardiness. It is mixed with the soil in spring, or incorporated in the compost. Taking in consideration the large concentration of oligoelements it is recommended to be used moderately, in a dosage of 1 kg/100 m^2.

Foliar fertilisation consists in vaporising fertiliser directly on to plant leaves, preferably on the underside of the leaves. The effect of this fertilisation method is very rapid, since the nutrients are immediately assimilated and used by the plants. Foliar fertilisation is particularly useful after plant stress (transplantation, hail, wind, cold, heat, and drought). Or in the case of severe nutritional deficiency, it is best applied as soon as the need is recognized.

Foliar fertiliser is better absorbed when vaporised into fine drops and applied early in the morning or in the evening. During these periods the air is cooler and more humid, favouring the opening of stomata.

The most common foliar fertilisers used are either liquid or soluble. Fertilisers based on algae, plants or fish are particularly effective and they are specifically suggested for short-term deficiencies in plants, and in commercial centres and flower shops where vegetation stagnates and plants don't flower due to low natural light, air currents, or plant refrigeration during transportation. The use of natural foliar fertilisers significantly cuts losses for this commercial segment between the producer and the final beneficiary.

Easley and Shirley (1974) consider that invasive aquatic plants should be harvested by mechanised means instead of treating them with specialised herbicides. This allows the plants to be acceptable for animal consumption. The study described the annual evolution of the mineral nutrient content in six macrophyte species sampled once and twice per month: *Hydrilla verticillata* (water thyme), *Eichhornia crassipes* (common water hyacinth), *Ceratophyllum demersum* (hornwort), *Potamogeton pectinatus* (sago pondweed), *Vallisneria americana* (eelgrass) and *Najas guadalupensis* (water nymph). Table number 2 presents the minimum and maximum concentrations of minerals in these plants compared to the daily needs for a 300 kg calf for the same elements. For Ca, Mg, Na, Fe and Mn these needs are largely met.

Table 2: Maximum and minimum concentrations of nutrients in aquatic macrophytes and percentages of the daily requirements of a 300 kg calf of the same nutrients contained in one kg of dry mass of macrophytes - according to Easley and Shirley (1974).

	% concentration of animal requirements in one kg dry weight								
Plants	Ca	P	K	Mg	Na	Fe	Cu	Zn	Mn
H. verticillata	300-600	10-26	50	100-160	100-200	70-140	63	33	100-200
E. crassipes	85	10-26	82	100-160	100-200	70-140	5-21	33	100-200
C. demersum	300-600	10-26	50	100-160	100-200	70-140	5-21	9-18	600-200
P. pectinatus	300-600	10-26	50	100-160	100-200	70-140	5-21	9-18	600-200
V. americana	300-600	10-26	25	50-80	100-200	68	5-21	9-18	100-200
N. guadalupensis	300-600	10-26	50	50-80	100-200	70-140	5-21	9-18	100-200

Shirley et al. (1971) analysed macrophytes of the genera *Hydrilla*, *Ceratophyllum*, *Potamogeton*, *Chara* and *Vallisneria* sampled from lakes, rivers and dikes. Linn et al. (1975) analysed the nutrient content of 21 aquatic plants. The authors concluded that these plants can be a useful food source for farm animals due to the high protein content and low, raw fibre content, thus indicating a high nutritional value. Also, the estimated contents of hemicelluloses, cellulose and lignin suggest that most of the macrophytes are highly digestible.

A fermentation study was performed on a mixture of 50% *Myriophyllum exalbescens*, 30% *Ceratophyllum*, 10% *Potamogeton pectinatus*, 5% *Vallisneria*, and 5% other aquatic plants. The mixture was fermented (siloed) in a laboratory setting using different organic acids and additives. Corn seeds and triturated alfalfa were also fermented in separate silos. The respective silos were analysed and measured and the following observations were made:

i. the dry matter in the aquatic plants silo was comparable with the one in the alfalfa silo, measuring 33.3 versus 32.3% respectively;

ii. all acid silos treated had less protein contents compared with the untreated ones, indicating a loss of protein mass through siloing;

iii. the ash content in the aquatic plant silo was high (47.5% in average);

iv. no major chemical discrepancies were recorded, including the organic matter content, when in the aquatic plant silos were added 5% corn or 75% alfalfa;

v. the formic acid was the most effective in reducing the occurrence of the butyric acid;

vi. adding alfalfa on sterilised aquatic plant silo led to the formation of a more acceptable silo than if alfalfa was added to unsterilized aquatic plants.

Little (1968 a, b) recommended the use of aquatic plants as fodder for pigs. These omnivorous animals have no problem accepting high water content in their food. Other authors reviewed by the FAO document tested aquatic plants in pig food and reported that the animals were reluctant to eat the plants as such, yet they accepted the aquatic plant ash mixed with other feed.

Other important substances highly concentrated in aquatic plants are the xanthophylls. Bailey (1965) mentions specifically *Ceratophyllum* sp., *Elodea densa* and *Myriophyllum exalbescens*. He states, “The feed ingredient industry is constantly alert for new ingredients or new additives which can improve the efficiency of the finished feed or can effect savings without detracting from the quality of the feed. In the above instance, we were impressed by the xanthophylls content of these dehydrated aquatic plants. Xanthophylls are oil-soluble carotenoid pigments found in some plants. There are many different xanthophylls. Some are effective in imparting a yellow colour to the skin of chickens and in darkening the yolk colour of eggs used mainly for the egg-breaking industry. Other xanthophylls are ineffective in this respect. Couch J. B. and associates of Texas A and M University recently reported that xanthophylls in these aquatic plants gave good colouration to egg yolks, and rated xanthophylls in aquatic plants about equal to that in alfalfa meal in this respect.” (in FAO Handbook).

Lange (1965) describes harvesting *Myriophyllum*, *Elodea* and *Ceratophyllum* in north-eastern Texas. The weeds were processed through a conventional alfalfa dehydration mill to produce 40 t of dried meal. This was sent to Texas A and M for a stock feeding programme which involved poultry, pigs and cattle. The results gave a strong indication that the potential for commercial production and marketing lay in the direction of a poultry feed supplement for egg yolk and broiler pigmentation. This was based on xanthophylls values in excess of 660 mg/kg, protein in the 20% range and a low fibre of 10-15%.

Regarding the use of *Myriophyllum spicatum* and filamentous algae, Bruhn et al. (1975) confirms that the birds are considerably efficient in assimilating proteins and xanthophylls from these plants.

Creger et al. (1963) analysed a dehydrated mixture of three macrophytes and concluded that it contained 17.9% proteins, 11% gross fibres and 440 mg xanthophylls/kg. Concentrations of 5, 10, 15, 20 and 25% administered in poultry fodder produced NEPA numbers of 3, 4.5, 5.5, 6.3 and 6.8 respectively when incorporated into milo-soybean oil meal basal diet and fed to laying hens. Levels of 10 and 20% of dehydrated alfalfa meal, which analysed 20% protein and 500 mg xanthophylls per kg, produced NEPA numbers of 4.75 and 6.1 under similar conditions. From these data it can be concluded that the naturally occurring xanthophylls found in a dehydrated blend of three species of flowering aquatic plants were biologically available and produced egg yolks of NEPA numbers similar to those which resulted from the feeding of approximately equivalent levels of xanthophylls from dehydrated alfalfa meal (FAO, 1979).

Linn et al. (1975) studied the digestibility rate in sheep for the same aquatic plants, both in a desiccated and fermented (siloed). They concluded that dried *Myriophyllum exalbescens* and *Potamogeton pectinatus* are not palatable for lambs (less than 600 g dry mass consumed daily).

The results of this study suggest the palatability of aquatic macrophytes can constitute a limiting factor in their use as fodder for ruminants. Desiccation and silo fermentation did not appear to be satisfactory methods in improving palatability. Unfortunately, this study was not confirmed by the findings of other scientists, its conclusions still need to be validated against other animal groups accustomed to consuming unfermented fodder.

Regarding economically important fish species that live in lakes or smaller European bodies of water, they all consume aquatic vegetal matter at least at one stage of their life cycle. Prejs (1984) listed 15 species, such as *Leuciscus idus*, *Rutilus rutilus* and *Scardinius erythrophthalmus* as being predominantly herbivorous; *Abramis brama*, *Blicca bjorkna*, *Leuciscus cephalus*, *Carassius carassius*, *Gobio gobio*, *Tinca tinca* and *Cyprinus carpio* consume vegetation in small quantities while *Rhodeus sericeus amarus*, *Alburnus alburnus*, *Perca fluviatilis* and juvenile *Esox lucius* are occasional consumers. Primarily herbivorous fish such as *R. rutilus* and *S. erythrophthalmus*, typically consume most of the submerged macrophytes found in large enough densities, with a preference for macrophyte species with soft tissues.

Bănăduc (2003), Bănăduc and Bănăduc (2008) and Bănăduc et al. (2011) revealed fish species in the Romanian national territory which include in their diets vegetals: *Alburnoides bipunctatus*, *Barbus meridionalis*, *Squalius cephalus*, *Romanogobio albipinnatus vladykovi*, *Barbus barbus*, *Chondrostoma nasus*, *Gobio gobio*, *Pseudorasbora parva* and *Rhodeus sericeus amarus*.

Ctenopharyngodon idella (snake-head fish) was introduced in Romanian fresh waters to help control water plants, but also for human consumption. In the United States after 1963 this species became very controversial due to the ecological consequences of its introduction. Research carried out in Europe and Asia, and in Petr (2000) starting in the 70's, documented that the *Potamogeton* species are a favourite food, or at least among the most consumed species of macrophytes in *C. idella*'s diet, as well as in the diet of his common carp hybrids and in the diet of *Hypophthalmichthys nobilis*. Mehta et al. (1976) in Petr (2000) demonstrated that in India, *Potamogeton* sp. is preferred by *C. idella* once the fish reaches at least 200 g.

Fowler and Robson (1978) in Petr (2000) mention cases in which *C. idella* (217 g average weight) forming fish stocks of 116-688 kg/ha went from a preferential diet of *Potamogeton crispus* to an unpreferential diet following the consumption of nearly the entire mass of *P. crispus* plants, the plant needing one year to recover once the fish was removed from the depleted reservoir.

Such uses for macrophytes, both field crop fertilisation and mulching, as well as food sources or supplements for farm animals from fish and poultry to pigs and bovines, generated remarkable economic results, which were well documented and recommended as Best Agricultural Practices by FAO since the 70's.

Ecological advantages of using aquatic macrophytes

On one side, the submerged aquatic macrophytes are substrata and habitat for incredibly complex and varied communities, but in uncontrolled conditions, a rich macrophytic lacustrine ecosystem has the tendency to self-regulate slowly, eventually evolving into overgrowing and warping.

On the other side, by the nature of vascular plants, the macrophytes of lotic ecosystems act as an efficient filter for nutrients of natural origin (excretion products and by-products), or artificial origin (fertilisers and pesticides historically administered in excess). In Bucharest more than 50-years-worth of these pesticides and fertilisers have reached the ground water and rivers that feed the urban lakes and the Colentina River, which are contaminated with sewage effluents introduced either intentionally or accidental by the metropolitan sewage network.

Howard-Williams (1981) describes an experiment in which 4.5 m^2 sized pans were planted in a thick *Potamogeton pectinatus* population and were supplied with weekly inputs of $NaNO_3$ and KH_2PO_4 in four different concentrations (max. 1,000 mg nitrogen/week and 100 mg phosphorus/week) during a period of 15-23 weeks.

The most obvious outcome was the development of dense populations of filamentous algae. Nevertheless, there was no parallel development of planktonic algae and the levels of chlorophyll remained low during the entire period of the experiment. Nine weeks later, for all levels of nutrients administered, the macrophytic community removed the entire administered quantity of nitrogen and phosphorus. Experiments with the isotope P_{32} demonstrated that a large part of the added phosphorus was being absorbed in two hours by the filamentous algae growing as epiphytes on the macrophytes. The nitrogen and phosphorus concentrations per unit of dry mass of algae, macrophytes and sediment increased significantly only for the most concentrated fertilising treatment.

The above mentioned experiment shows that the areas with a large density of submerged aquatic macrophytes, together with their associated epiphytic algae (mainly *Cladophora* sp.), are very efficient and useful nutrient filters for the purification of stagnant or slowly running surface waters.

Scarsbrook and Davis (1971) measured the growth of five aquatic plants in well water to which sewage effluent had been added; this was recorded together with their uptake of nitrogen, phosphorus and potassium. The plants were *Eichhornia crassipes* (water hyacinth), *Alternanthera philoxeroides*, *Potamogeton crispus*, *Egeria densa* and *Najas flexilis.* The tests were in plastic pools 66 cm deep and 2.7 m in diameter, containing no soil. A mixture of all the plants was put in each pool. Half the pools had 25% sewage effluent, the others had well water only. In the latter pools all the test plants died except *A. philoxeroides*, but its growth was stunted and unhealthy. In the sewage-treated pools, by the end of 11 weeks the water hyacinth had dominated all the other species and covered 71% of the water surface. The next vigorous level was *A. philoxeroides*, though its growth was much less than that of the water hyacinth. The other test plants did not grow sufficiently in the face of the competition to justify harvesting. The results are shown in table number 3.

“The authors concluded that water hyacinth could be usefully employed to extract nutrients from sewage.” (FAO, 1979).

The following results from the survey of water quality in the Tineretului Lake of Bucharest, Romania come from the period of October 2007 to August 2008, which includes the vegetation season of the aquatic macrophytes (*Potamogeton crispus* mainly and *Myriophyllum spicatum*) and the harvesting of the entire macrophyte community during June and July 2008. These results have also been largely presented in Hurdugan-Irimia (2008).

Table 3: Growth over 23 weeks (April-October - g dry weight) of five aquatic plant species treated with well water and sewage effluent. From Scarsbrook and Davis (1971) in FAO (1979); includes weight of plants harvested at 11 weeks.

	Initial weight	Harvested weight	
Plant		Well water only	25% sewage effluent
E. crassipes	2.0	59.0	736.6
A. philoxeroides	0.6	7.9	20.4
P. crispus	0.5	0.9	4.7
E. densa	0.2	0.0	0.0
N. flexilis	0.1	0.0	0.0

During the maximum vegetation season the differences between the source area and the outlet point are dramatic, with up to a 10-fold decrease of the CO_2 concentration in the water. These differences disappeared when the vegetation was removed.

The oxygenating effect of the macrophytes and of the epiphytic filamentous algae is visible in the difference between the 8 mg/l concentration of the dissolved oxygen in the sources area (at the origin of the lake) and 12.5 mg/l at the outlet point during the vegetation period. The same parameters registered 10.5 mg/l dissolved oxygen in the sources area versus 11 mg/l in the outlet point once the vegetation was removed.

The same goes for the absorption of the CO_2 from the lake water by the macrophytes and the epiphytes in the analysed period. There was also a decrease of the concentration of carbonate ion in the water through the effect of the biological pump of CO_2 modifying the balance of the naturally present carbonates in the lake. The pump effect increased the supplementary support offered by the plants, especially *P. crispus*, for the deposit of the Ca and Mg carbonates on the macrophytes.

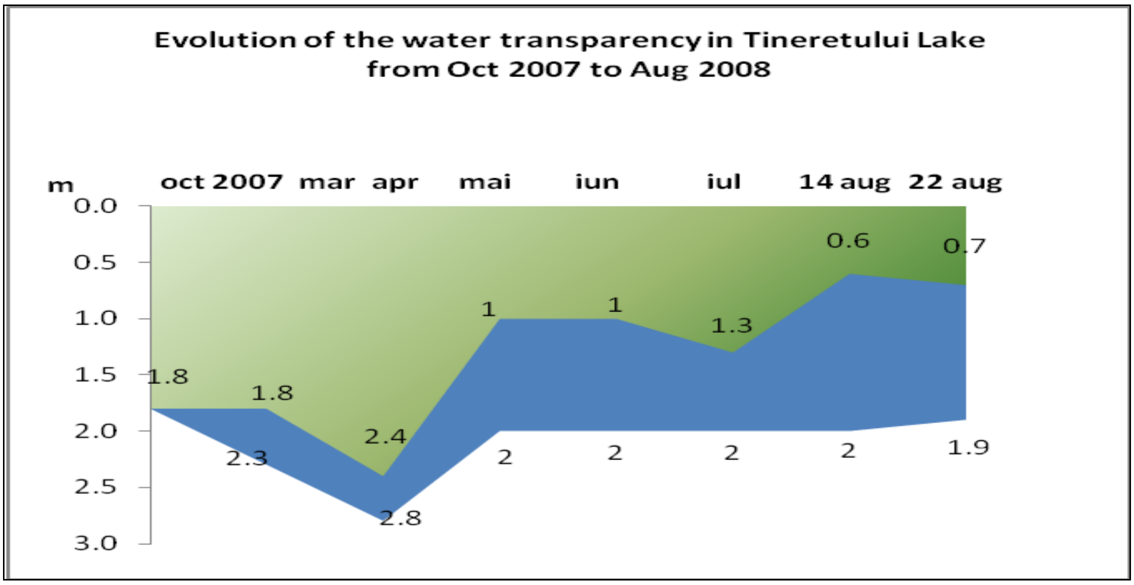

Figure 1: The water transparency evolution in the Tineretului Lake during October 2007 - August 2008. The green polygon represents the Secchi disk measured transparency. The sum of the green and blue polygons represents the measured depth in the sampling point (Hurdugan-Irimia, 2008).

Regarding nitrates, nitrites and ammonia, the main excretion products of the lake fauna, there is a clear discrepancy between the constant concentrations of these products in the lake water during the period before the harvest of the macrophytes, followed by a large increase of the same parameters' values after the harvest; up to three times more than the regular concentration. Also, differences between the sources area of the lake and the outlet point are noticeable in the concentrations of nitrogen compounds (from 0.8 mg/l nitrates in the sources area to 0.5 mg/l at the outlet and from 89 mg/l in the sources area to 50 mg/l at the outlet) during the vegetation season, marking the importance of the macrophyte role in water purification.

The ammonia is not directly taken up by macrophytes but it is decomposed by *Nitromonas* bacteria into nitrites. Its concentration remained constant from the origin to the outlet of the lake, yet it varied in time, reaching dangerous levels for fish after the removal of the submerged macrophytes.

"All the analyzed parameters, nitrites, nitrates, dissolved oxygen and carbon dioxide, ammonia and transparency (Fig. 1) have presented optimum values for the March-April period, correlated with the period of macrophytic vegetation growth which reached its maximum development in height and density at the end of April. After the harvesting period from May 2008, the water quality deteriorated significantly and the phytoplankton received the necessary light, temperature and nutrient stimulation in order to bloom massively. The evolution of the physical and chemical indicators of the water quality have closely followed the presence of the macrophytic species, especially that of the *Potamogeton crispus*." (Hurdugan-Irimia, 2008)

Harvest and elimination - biological control

The use of rakes, chains, and mechanical reapers to remove the submerged aquatic vegetation is known to be both costly and laborious, yet the results are not considered satisfactory. For instance, mechanised controls generally engendered thicker plant populations during the next season due to the reduced competition for nutrients and light, according to Neel et al. (1973) in Petr (2000). Also mechanical removal leads to the occurrence of morphologically smaller individuals (Engel, 1984, in Petr, 2000) a phenomenon personally witnessed in August 2008 in Tineretului Lake, Bucharest.

Due to the hydrochoric spreading of these plants, their cutting and harvesting, even up to three times in a year, will never completely and definitively remove the species from the ecosystem.

Nature has a way, *Ctenopharyngodon idella* is the fetish species of phytophagous fish introduced in still waters for the biological control of the biomass of submerged macrophytes. According to research led by Mitzner in 1978, in Petr (2000), this fish species was responsible for the reduction of the July biomass in *Potamogeton crispus* and *Potamogeton nodosus* from 1,400 g dry weight/m^2 to 24 g dry weight/m^2 in four years, but three years later, the *C. idella* biomass decreased to the level of the second year of introduction.

Ctenopharyngodon idella is a voracious consumer of *Potamogeton*, this macrophyte species being its favourite in pilot farms. Yet they need to be controlled by predators in order not to exterminate the plants completely and degrade the local ecosystem since the partial control of the plants is very difficult to obtain due to the gluttony of the snakehead carp.

Since the adverse effects of the *Ctenopharyngodon idella* were noticed after its introduction in lakes dedicated to sport fishing and leisure, mainly due to overpopulation, research concentrated on the use of sterile diploids and triploids of *Ctenopharyngodon idella* in order to control aquatic macrophytes (Bowers et al., 1987, in Petr, 2000). Besides, these fish retain up to 90% of the dissolved phosphorus from administered pesticides and fertilisers present in the water according to Chapman and Green (1987). Wiley et al. (1984) created special computer models of fishery management demonstrating that the triploid *Ctenopharyngodon idella* is economically more efficient in controlling aquatic vegetation than the use of pesticides.

Other species that can be used to control macrophytes are waterfowl, wild or domestic: ducks, geese, as well as swans and moorhens, all of which are present in large numbers in Bucharest lakes. For instance, the author personally identified in 2006-2008 around 50 pairs of mallards *Anas plathyrhynchos* on Tineretului Lake in central Bucharest alone, as well as a family of nine mute swans *Cygnus olor*, and four pairs of moorhens *Fulica atra*, etc. All these species were consuming large quantities of aquatic vegetation, especially soft-tissues plants. Other waterfowl species consume the grains and/or fruits of aquatic macrophytes, thus controlling their spread, especially for Potamogetonaceae, Cyperaceae and Polygonaceae, Potamogetonaceae being the favoured food source. Grazing significantly affects biomass, as well as the growth of submerged macrophytes.

Waterfowl are particularly efficient in the control of aquatic macrophyte biomass, reaching consumptions up to 40% of the live biomass of *Potamogeton pectinatus* in Manitoba marshy delta, Canada (Anderson and Low, 1976, in Petr, 2000), 30% of the live biomass of *P. filiformis* in Loch Leven, Scotland (Jupp and Spence, 1977, in Petr, 2000) and around 50% of the live biomass of a mixed assemblage of submerged macrophytes in Tipper Grund, Denmark (Kiorboe, 1980, in Petr, 2000).

The success of waterfowl in controlling the aquatic macrophytes is also demonstrated by figure number 2 a, b, showing the macrophyte consumption ratios for rudd (*S. erythrophthalmus)* and waterfowl in Zwemlust Lake, Holland, where the estimated annual consummation of macrophytes by coot (*Fulica atra*) was calculated as 30 to 1,200 kg/year, compared to the rudd, situated between 170 and 360 kg/year (Van Donk and Otte, 1996, in Petr, 2000). Mitchell and Perrow (1998) suggest that the birds have a major impact on the dynamics of the aquatic vegetal biomass in temperate areas only during periods of weak biomass production, either in early spring when the biomass is still at low levels, or late in the autumn when the primary production slows its rhythm or stops. This was noticed by Olofsson (1991) for *Potamogeton crispus* in the case of Stigholm Lake of Denmark.

These “gentle” methods to control the macrophyte biomass produced in a lake are preferable to mechanised or chemical controls. “Gentle” methods of control are part of the old and long-tested mechanisms of Nature, which always tend toward the optimum regulation of physical, chemical or biological resources. These methods permit the maintenance of a particularly clean lake (in terms of transparency, purity, richness, biological diversity) as a whole. Additionally, these methods are also the cheapest and the most ecological and aesthetically pleasing.

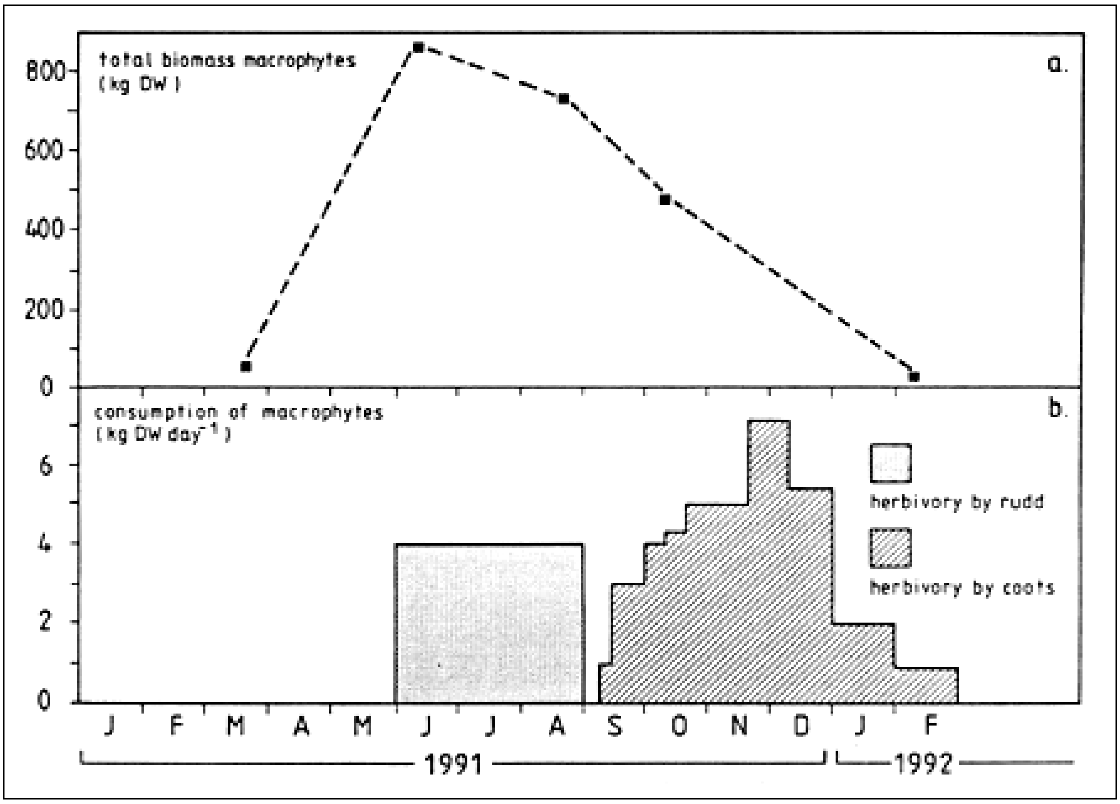

Figure 2: a) Total biomass of aquatic macrophytes (dry weight kg) in Zwemlust Lake estimated in March, June, August, October 1991 and February 1992;
b) Estimations of the consummation (dry weight kg of macrophytes/day) by rudd and coots in Zwemlust Lake (May 1991 - February 1992) from Van Donk et al. (1994).

CONCLUSIONS

Presently, the management of the lakes in the area of Bucharest oscillate between two alternatives. At one end of the scale are lakes intensively used for fishing, administered according to a pseudo-economical logic excluding the ecological reasoning and means, in which the ecosystems are out of balance and frail. One such example is Herăstrău Lake, where during the hot summer of 2007, the economically interesting populations of fish were severely affected by hypoxia and the lake water registered toxic levels of ammonia and nitrogen compounds (personal observations) while zooplankton invertebrates disappeared very early in the season (almost azoic, negative for Cladocerans samples in October 2007). At the other end of the scale are lakes abandoned to the good work of Nature, such as Văcăreşti, an oasis for waterfowl and other water-related fauna, which are threatening to disappear due to real-estate projects and the natural overgrowth of reed and cattails.

Somewhere in the middle are lakes whose management, during certain periods of time, were cared for through respectful and ecological approaches. For example Tineretului Lake during 2004-2007. Here, the fishery and leisure facilities practiced sustainable development. The lake water had a superior quality compared to tap water during winter, and the ecosystem was balanced and stable. It was an oasis for 23 species of fish and birds protected by the Bern Convention and CITES Convention. Not to mention the lake is also home to 48 species of phytoplankton, 46 species of epiphytes, two species of macrophytes, 15 species of cladocerans, at least six species of aquatic insects, two species of molluscs, nine species of fish, three species of amphibians, two species of chelonians, and 19 species of birds (Hurdugan-Irimia, 2013). During the same reference period for Herăstrău Lake (July-October 2007), Tineretului Lake zooplankton were numerous, the water had a good quality and the fish population was not affected by heat, nor a lack of oxygen (personal observations).

The same lake stands as proof of the negative effects that the total elimination of the submerged macrophytes can have. After the harvest in May 2008, the water quality deteriorated rapidly and the phytoplankton, stimulated by light, high temperatures and lack of competition for nutrients, had a massive bloom. This food source was not appropriate for the ducklings that year, many of which died (more than 50% mortality for some mallard families that year, according to the observations of the lake administration). Also, due to the lack of the shelter usually provided by the macrophytes at that time of year, many of the chicks and ducklings were attacked by pike and catfish (near 30% mortality for some families, according to the observations of the lake administration).

The submerged aquatic macrophytes are a part of the lacustrine ecosystems, both a cause and a symptom of their quality. A lotic ecosystem, whose equilibrium is characterised by a high degree of homeostasis, requires a reasonable surface to be occupied by aquatic vegetation in order to support the macrophytophylous species, either invertebrates or economically interesting fish and waterfowl.

Considering that the ground water nape and surface waters that feed into the fishing waters of the Câmpia Română are heavily charged with nitrates from 50 years of intensive agriculture, such surface coverage is indispensible for maintaining proper water quality.

The vegetation patches can also contribute significantly to an aesthetic look of the water body, providing a pleasant sight as well as transparent, clean water and a rich fauna worthy of a civilised European metropolis, which the capital city of Romania aspires to.

If we consider all of these reasons, including a larger production of fish of all species in the lake, an economical valorisation of the harvested macrophytes from two-thirds of the lake surface for agriculture, horticulture and animal rearing, one would observe a proper example of integrated, successful management of urban lakes. In doing so, the logic shifts from winner-loser, to everybody wins way of thinking: the Lakes, Parks and Leisure Facilities Administration, the environment, the population, the city image and the future generations.

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LONG- AND SHORT-TERM CHANGES OF THE STRUCTURE OF MACROPHYTES IN LAKE PIASECZNO IN RELATION TO LAND USE IN THE ŁĘCZNA-WŁODAWA LAKELAND (POLAND)

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DOI: 10.2478/trser-2013-0022

KEYWORDS: Poland, Łęczna-Włodawa Plain, land-use changes dynamics, lakes, macrophytes.

ABSTRACT

The Łęczna-Włodawa Plain, known also as the Łęczna-Wlodawa Lakeland, lies within the territory of the largest subregion of the Polesie region, covering over 1,300 km^2. The main interest of the Łęczna-Włodawa Lakeland is that it is the oldest in the Central European Lowlands group of about 68 lakes. Among such a large number of lakes there exist all trophic types. However, since the late 1950’s, enormous dynamics of change associated with the disappearance of oligo- and mesotrophic lakes and their transformation into eutrophic, even hypertrophic, lakes have been observed. One of the biocenotic elements of the lakes, which are indicators of these changes, is aquatic plants. The aim of this study was to determine the macrophyte structure of Piaseczno Lake and changes of land use in its surroundings. Piaseczno Lake still represents very high natural values. A reduction in the number of macrophyte communities, which occurred especially in 2008, was a consequence of the fast-growing recreation infrastructure.

From 1976 until 2010 an area of recreation infrastructure in the studied area increased more than 3.5 times, and in the built-up area more than five times, as well as a doubling of the total length of the roads. Meanwhile the surface area of wetlands and peatbogs significantly decreased - more than 11 times.

Long-term changes in the structure of the macrophyte communities show that the number of communities has varied in each year, probably as a consequence of changes in land-use. Analysis showed changes to the surfaces inhabited by macrophytes, which have decreased significantly over only four years, by more than 25%. However, the proportion of rush communities has increased.

RÉSUMÉ: Changements à court et long terme dans la structure des macrophytes du Lac Piaseczno en rapport avec les modifications de l’utilisation des terrains dans la région des lacs de Łęczna-Włodawa (Pologne).

La Plaine de Łęczna-Włodawa, également nommée la région des lacs de Łęczna-Wlodawa, est la plus grande subdivision de la région de Polésie couvrant plus de 1.300 km^2. L’intérêt principal de la région des lacs de Łęczna-Włodawa Lakeland est le plus ancien groupe de lacs, environ 68, de la plaine centrale européenne. Un nombre aussi important de lacs laisse présager différents types trophiques. Cependant, depuis la fin des années 50, une transformation des lacs oligo- et mesotrophes en lacs eutrophes voir hypereutrophes est observée. Un des éléments de la biocénose lacustre indiquant ces modifications est la flore macrophytique. De ce fait, l'objet de cette étude a été la détermination de la structure des macrophytes du Lac Piaseczno ainsi que les changements de l'utilisation des terrains environnants. Le Lac Piaseczno présente malgré tout une grande valeur naturelle. La réduction du nombre des communautés macrophytiques, un phenomène qui s'est manifesté surtout en 2008, a été la conséquence de la croissance rapide de l'infrastructure récréationelle.

Entre 1976 et 2010, la superficie de l'une des aires d'infrastructure récréationnelle de la zone étudiée s'est agrandi de plus de 3,5 fois, la zone construite plus de cinq fois et les routes couvrent désormais une surface deux fois plus importante que dans les années 70. En parallèle, la surface des zones humides et des tourbières a diminuée de manière significative plus de 11 fois.

L'observation des changements à long terme de la structure des communautés macrophytiques a montré que leurs nombres varient chaque année. Ce phénomène est probablement dû à une utilisation différentielle des terrains. En effet, l'analyse des modifications de la surface occupée par les macrophytes montre de manière significative une diminution de plus de 25% de leurs nombres durant les quatre dernières années. En revanche, la proportion de joncs a augmenté.

REZUMAT: Modificările pe termen scurt și lung în structura macrofitelor din Lacul Piaseczno în urma schimbărilor în utilizarea terenurilor din regiunea lacustră Łęczna-Włodawa (Polonia).

Câmpia Łęczna-Włodawa, denumită și regiunea lacustră Łęczna-Wlodawa este cea mai mare subdiviziune a regiunii Polesiei, cu o suprafață de peste 1.300 km^2. Atracția principală a regiunii lacustre Łęczna-Włodawa este un grup de circa 68 lacuri, cel mai vechi ansamblu lacustru din Câmpia Europei Centrale. Având în vedere numărul mare de lacuri, aici se găsesc toate tipurile trofice de acvatorii dulcicole. Însă de la sfârșitul anilor '50 dinamica schimbărilor a devenit foarte importantă, fiind asociată cu dispariția lacurilor oligo- și mezotrofe și transformarea lor în lacuri eutrofe și chiar hipertrofe. Unul din elementele biocenozei lacustre, indicator al acestor modificări, este flora acvatică. Scopul prezentului studiu a fost determinarea structurii macrofitice a lacului Piaseczno și a schimbărilor în utilizarea terenurilor din vecinătate. Lacul Piaseczno încă mai reprezintă o valoare naturală importantă. Reducerea numărului de comunități macrofitice, apărută în special în 2008, a fost o consecință a creșterii rapide a infrastructurii recreaționale.

Între 1976 și 2010, una din ariile de infrastructură recreațională din zona studiată a crescut de peste 3,5 ori, iar zona construită a crescut de cinci ori în timp ce suprafața drumurilor a crescut de două ori. În același timp, suprafața zonelor umede și a turbăriilor a scăzut semnificativ - de peste 11 ori.

Modificările pe termen lung în structura comunităților de macrofite au arătat că numărul comunităților a variat de la an la an, probabil în urma modificărilor în utilizarea terenurilor. Analiza modificărilor suprafeței ocupate de macrofite arată că aceasta a scăzut semnificativ în decurs de numai patru ani, cu peste 25%. În schimb, a crescut procentul ocupat de comunitățile de *Juncus* sp.

INTRODUCTION

Lakes are natural depressions filled with water, which have no direct connection to the sea and have a minimum surface area of 1 ha (Stańczykowska, 1990; Bajkiewicz-Grabowska and Mikulski, 1999).

One of the processes that all natural waters are subject to, regardless of human activity is eutrophication. Eutrophication is an increase of fertility caused by an increasing concentration of minerals (P, N and C) and some environmental and biological changes in water quality. Excessive fertility of aquatic ecosystems results from human activities, such as sewage discharges, agricultural intensification or deforestation in the catchment (Stańczykowska, 1990).

Recently, in Poland and in the world, the majority of lakes are eutrophic lakes - fertile reservoirs, with a high content of salt nutrients and organic matter. Generally, they are relatively shallow (6-24 m). At the bottom levels of the water oxygen deficits are common. Sedimentary processes outweigh the schedule. The color of the water is green and yellow, with little transparency. The number of the phytoplankton species is high and blooms are frequent, and the littoral zone is strongly developed in them as well. The coastal vegetation is abundant. Animal plankton is also rich in species. Benthic fauna, represented by only few species, but simultaneously by a large number of individuals, are adapted to live in conditions with a low oxygen level. Eutrophic lakes can turn into low peatbogs or disappear. The rate of their evolution is very different and depends on the intensity of the flow and the quality of allochtonous matter, as well on the catchment management. In the period of about 11,000 years that have elapsed since the creation of most of the glacial lakes in Poland, about two-thirds of the total surface area has already disappeared (Stańczykowska, 1990). The phenomenon of lake disappearance also occurs in lakes of the Łęczna-Włodawa Lakeland (Chmielewski, 2009).

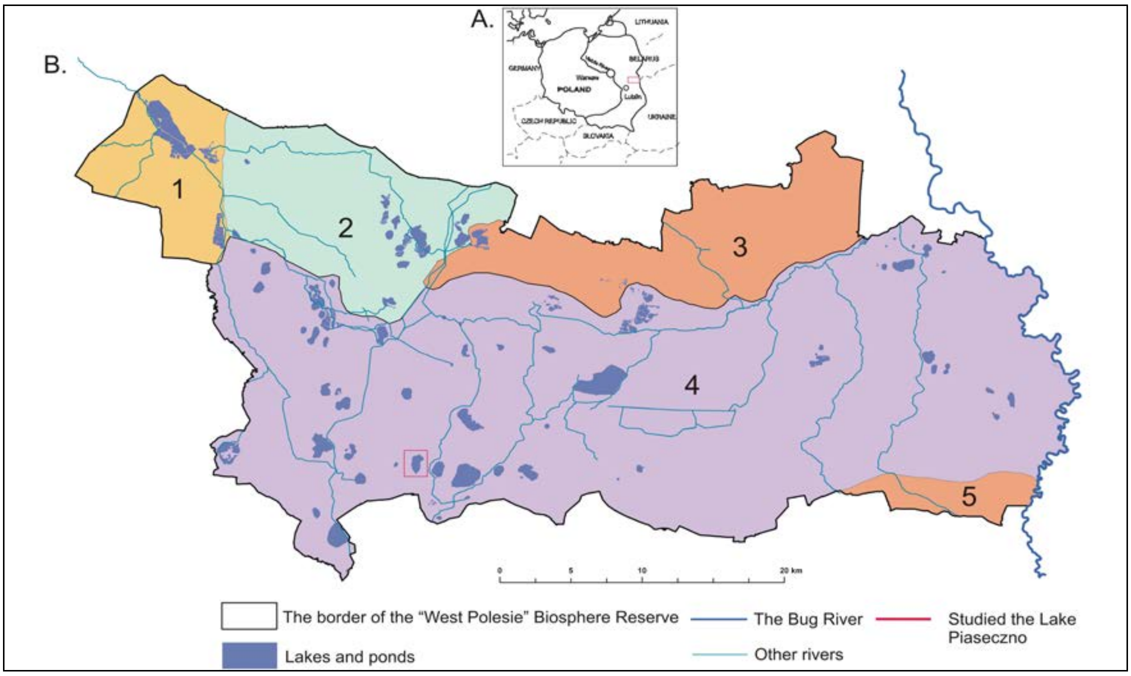

Figure 1: A) Location of the study area in Poland. B) The Łęczna-Włodawa Plain and its surroundings at the borders of the "West Polesie" Biosphere Reserve:
1 - Lubartów Upland (319 m above the sea), 2 - Sosnowica Depression (845 m above the sea), 3 - Włodawa Hummock (845 m above the sea), 4 - Łęczna-Włodawa Plain,
5 - Chełm Hill (845 m above the sea) (Kondracki, 1994).

The Łęczna-Włodawa Plain, called also as the Łęczna-Wlodawa Lakeland is the largest subregion of the Polesie, covering over 1,300 km^2. It is a former, wide river valley, in which after the end of the last glaciations, the water of large hollows started running down. It simultaneously concentrates vast swamps and a group of several dozen lakes. It is one of the most valuable regions in Poland, where in 2002 the "West Polesie" Biosphere Reserve was created (143,937 ha). In the central part, it contains the Polesie National Park, which is surrounded by three landscape parks (Łęczna Lakeland, Polesie and along the Bug River - Sobibór landscape parks), 12 nature reserves (among them seven on the Łęczna-Włodawa Plain) and 21 Natura 2000 sites (Figs. 1 and 2).

The river and stream network is quite dense (belong to the Wieprz and Bug River catchments), but because of the terrain's flatness, water flow is low or even minimal (Wójcikowski, 2006).

The main attraction of the Łęczna-Włodawa Lakeland area is the fact that is the oldest in the Central European Lowlands group of lakes, formed of about 68 lakes. There are both glacial lakes and lakes with karst origin. The largest lake is Uściwierz Lake (284 ha), and the deepest lake is Piaseczno Lake (about 39 m) (Harasimiuk et al., 1998). Among such a large number of lakes there are all trophic types. However, since the late 1950's an enormous dynamic of changes associated with disappearance of oligo- and mesotrophic lakes and their transformation into eutrophic lakes and even hypertrophic lakes is observed. One of the lakes' biocenotic elements, which are indicators of these changes, are aquatic plants. They are a stable component of lakes, reacting to adverse changes in its habitat (Sender, 2009, 2012). The aim of the study was to determine macrophytes structure of the Piaseczno Lake and changes of land use in its surroundings.

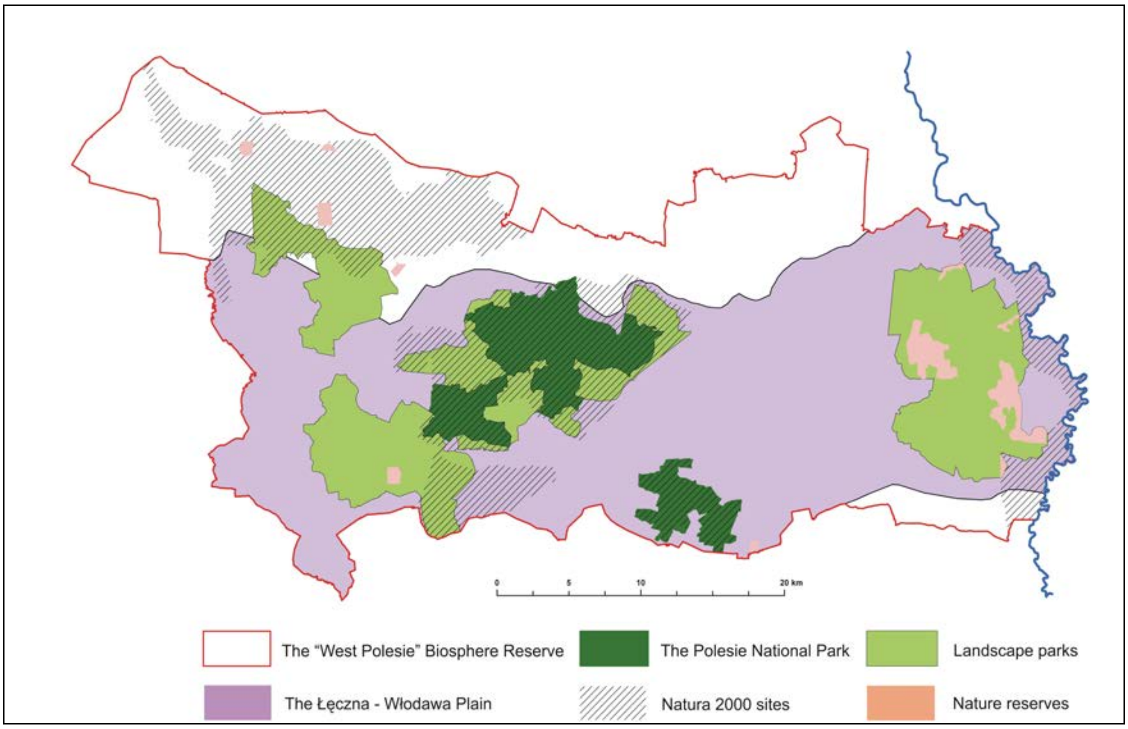

Figure 2: Network of protected areas in the Łęczna-Włodawa Plain, on the background of the "West Polesie" Biosphere Reserve.

STUDY AREA AND METHODS

Piaseczno Lake is located in the south-west part of the Łęczna-Włodawa Lakeland. It doesn’t have an outflow of surface water (Fig. 3, Tab. 1).

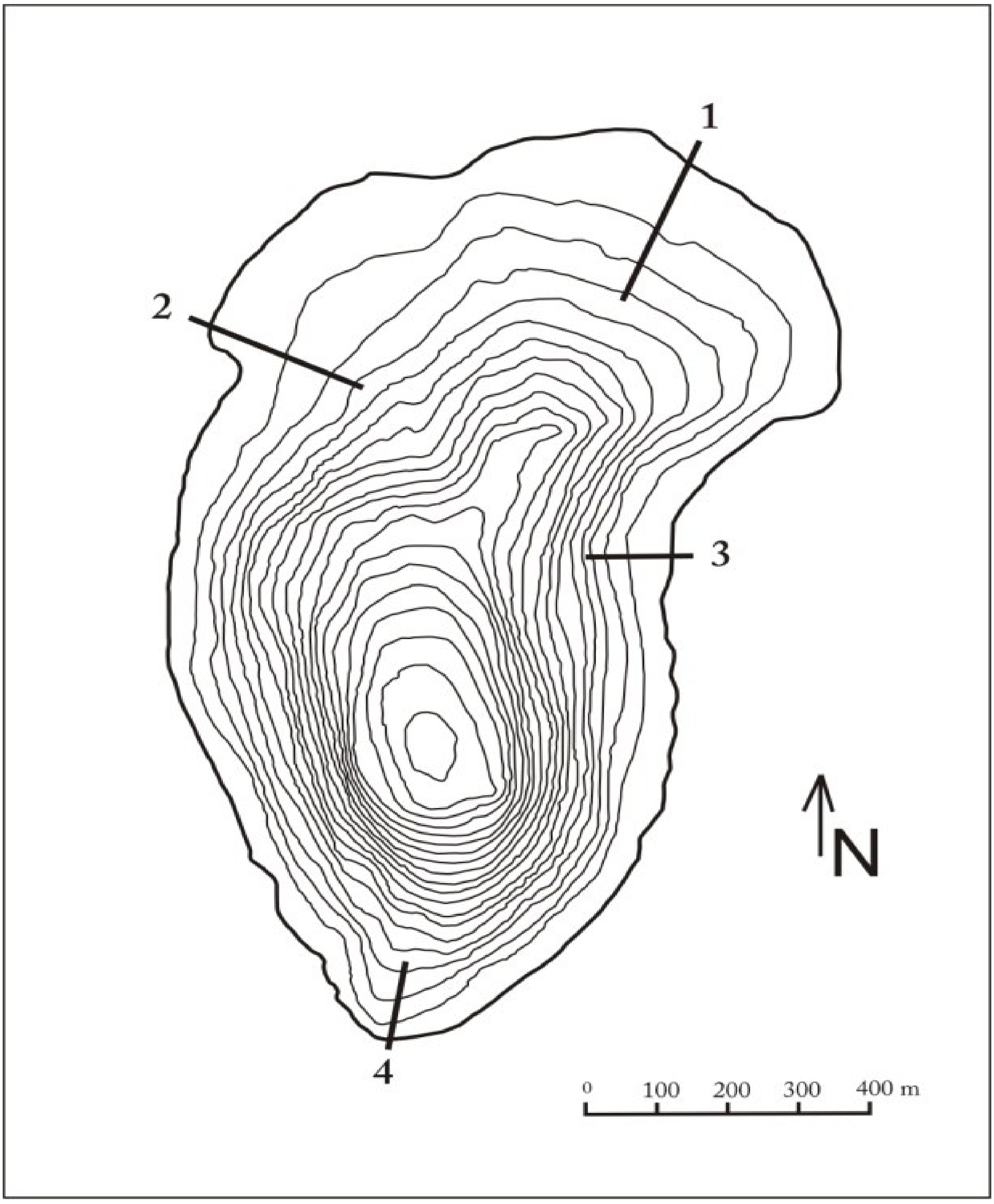

Figure 3: Distribution of research transects in the Piaseczno Lake;
1 - re-creation of ordered water sewage services, 2 - peatlands,
3 - re-creation of not ordered water sewage services, 4 - forest.

Table 1: Some morphological parameters of the investigated lake.

Water surface (ha)	84.7
Capacity (tys. m^2)	10.674
Depth (m)	38.8
Average depth (m)	12.6
Length of shore line (m)	3.788

In the 1950's it occurred as an oligotrophic lake, however actually it is defined as a mesotrophic lake, and some of its features may even suggest an eutrophic character nowadays. At a distance of about 700 m from the lake there is a water-bog reserve called Brzeziczno Lake, which contains many rare northern and Atlantic plants. The lake water is used for fishery. Due to the type of fishing, the lake was considered as a common bream and vendac type lake. It is restocked with eel, pike, crucian carp and common bream. Piaseczno Lake is situated in the catchment of the South Piwonia River. The catchment's surface area is 284.88 ha, out of which 29% constitutes agricultural lands, and 24% constitutes forests (Harasimiuk et al., 1998).

Phytosociological studies were carried out in the years 2008 and 2012 with the generally accepted phytosociological method (Braun-Blanquet, 1951). Phytosociological units (determined by the dominant species) were distinguished using a systematic and nomenclature system by Matuszkiewicz (2005). Trials of emergent and submerged macrophytes were collected along profiles highlighted after the inventory in the field (Fig. 3) (Bernatowicz, 1960; Szmeja, 2006). Based on the analysis of aerial photographs from the years 1976, 1984 and 2010, an analysis of the direct land use of Piaseczno Lake catchment was carried out. For the analysis Arc Gis 10.1 software was used.

RESULTS AND DISCUSSION

Direct land uses of the Piaseczno Lake catchment area are: forests, agricultural land, scrubs and forests, wetlands and recreational zones with built-up areas. During the analysis period of the study, there was an increase of areas occupied by forests and scrubs, as well as recreational areas and associated buildings and roads. A significant increase of recreational areas resulted in a significant reduction of wetlands, where the water extraction was related with drainage. From the point of view of the functioning of the catchment as a biofilter, such changes are very unfavorable, especially for peat bogs in which drainage causes the phenomenon of decay of peat and water penetration by the vast amounts of matter, accelerating eutrophication processes (Stachurski and Zimka, 1994). Also, agricultural land decreased, mainly because of allocation for recreation. The water surface remained at a stable level with a tendency to increase in recent years (Figs. 4 and 5).

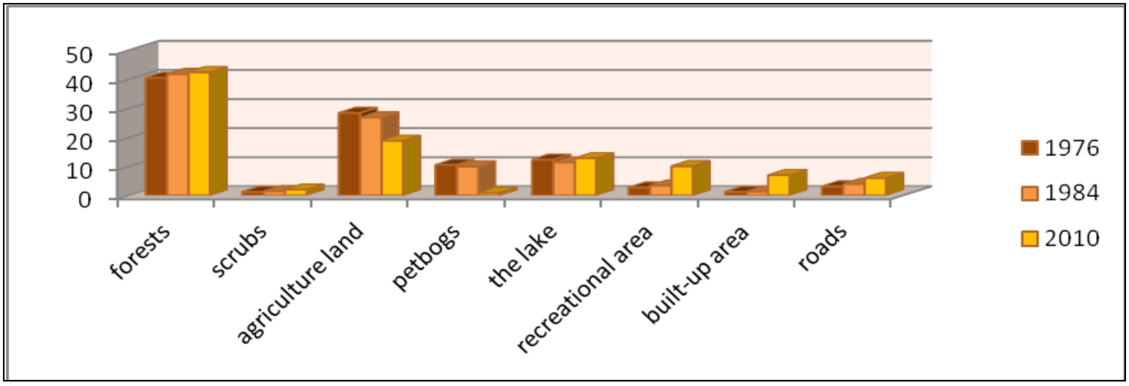

Figure 4: Land use changes in the surroundings of the Piaseczno Lake in 1976, 1984, 2010.

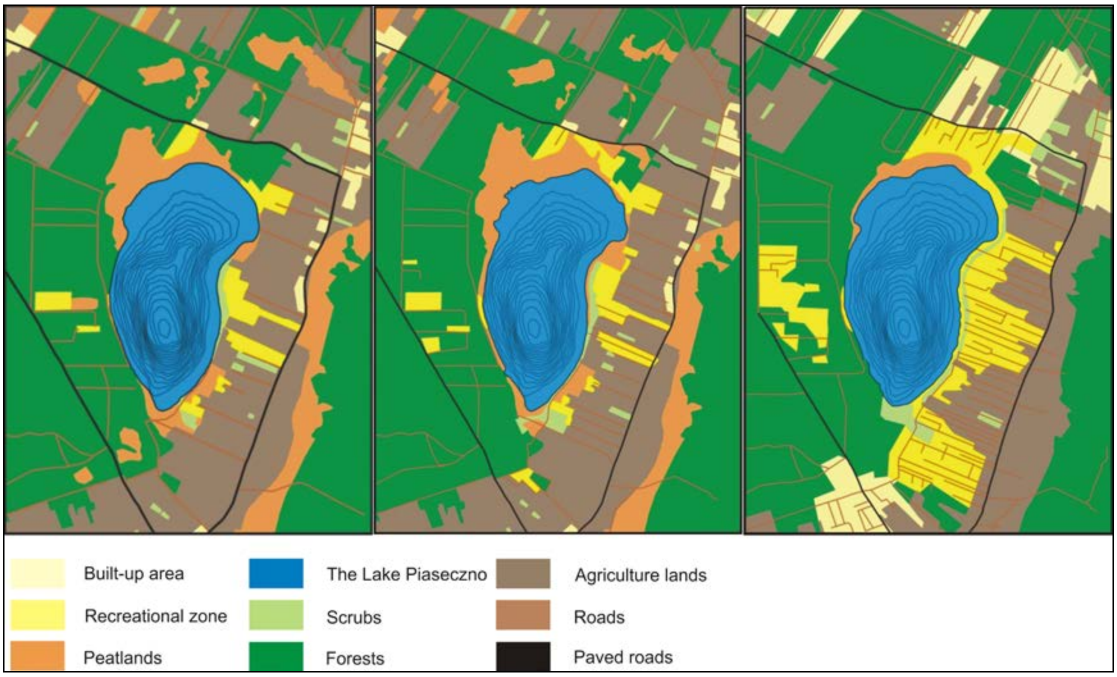

Figure 5: Land use changes around Piaseczno Lake in 1976, 1984, 2010 (percentage share).

Littoral zone was inhabited by 14 macrophytes communities in 2012. The total surface inhabited by macrophytes amounted to 25.3 ha, among it 17 ha constituted communities of submerged plants, whereas 8.3 ha of emergent plants (Tab. 2).

Table 2: Area covered by main types of plant communities in the studied lake in 2012.

Community	Area (ha)
Submerged plants	17.005
Community with *Utricularia vulgaris*	0.046
Community with *Chara delicatula*	0.685
Nitelletum flexilis	0.169
Charetum fragilis	1.6
Myriophyllo-Littorelletum	12.33
Ceratophylletum demersis	1.667
Elodeetum canadensis	0.508
Emergent plants	8.283
Epilobio-Juncetum effusi	0.041
Salicetum pentandro - cinereae	0.6619
Caricetum rostratae	0.028
Typhetum latifoliae	0.093
Typhetum angustifiliae	0.029
Eleocharitetum palustris	0.154
Phragmites australis	7.276
Total	25.288

The plant communities' number occurring in Piaseczno Lake was subjected to significant changes. At the end of 1950's, the lake under study was inhabited by 12 plant communities. In 2008 only ten plant communities were noticed, however, currently there are 14 (Tab. 3).

Table 3: Plant communities of the Piaseczno Lake in selected periods of research (Fijałkowski, 1959).

Community	Years		
	1960/70*	2008	2012
Cl. Charetea (Fukarek 1961 n.n.) Krausch 1964; O. Charetalia fragilis Sauer 1937; Ass. Charion fragilis Krausch 1964			
Charetum fragilis Fijałkowski 1960	+	+	+
Charetum asperae Corillion 1957	+		
Community with *Chara delicatula* Desev.	+	+	+
Nitelletum flexilis Corillion 1957	+	+	+
Cl. Potametea R. Tx. et Prsg 1964; O. Potametalia Koch 1926; Ass. Potamion Koch 1926 em.			
Elodeetum canadensis (Ping. 1953) Pass. 1964	+	+	+
Potametum lucentis Hueck 1931	+		
Potametum perfoliati Koch 1926	+		
Ceratophylletum demersi Hild. 1956		+	+
Cl. Littorelletea uniflorae Br. - Bl. et R. Tx. 1943; O. Littorelletalia uniflorae Koch 1926; Ass. Lobelion (Vanden Berghen 1944) R. Tx. et Dierss. ap. Dierss 1972			
Myriophyllo-Littorelletum Jaschke 1959	+	+	+
Cl. Phragmitetea R. Tx. et Prsg 1942; O. Phragmitetalia Koch 1926; Ass. Phragmition Koch 1926			
Eleocharitetum palustris Sennikov 1919	+	+	+
Phragmitetum australis (Gams 1927) Schmale 1939	+	+	+
Typhetum angustifiliae (Allorge 1922) Soó 1927	+		+
Typhetum latifoliae Soó 1927			+
Caricetum rostratae Rübel 1912			+
Scirpetum lacustris (All. Chouard 1924)	+		
Cl. Scheuchzerio-Caricetea (Nordh. 1937) R.Tx. 1937; O. Scheuchzerietalia palustris Nordh. 1937; Ass. Caricion lasiocarpae Vanden Ber. ap. Lebrun et al., 1949			
Community with *Juncus articulatus* L.		+	
Cl. Utricularietea intermedio - minoris Den Hartoget Sedal 1964 em. Pietsch 1965; O. Utracularietalia intermedio - minoris Pietsch 1965; Ass. Sphagno - Utricularion Mull. et. Gors			
Community with *Utricularia vulgaris* L.		+	+
Cl. Molinio-Arrhenatheretea R. Tx. 1937; O. Molinietalia caeruleae W. Koch 1926 Ass. Calthion palustris R. Tx. 1936 em. Oberd. 1957			
Epilobio-Juncetum effusi Oberd 1957			+
Cl. Alnetea glutinosae Br.-Bl. and R. Tx. 1043; O. Alnetalia glutinosae R. Tx. 1937; Ass. Alnion glutinosae (Malc. 1929) Meijer Drees 1936			
Salicetum pentandro-cinereae (Almq. 1929) Pass.			+
Total	12	10	14

Such significant fluctuations in the number of macrophyte communities inhabiting the lake are probably connected with changes in land use in the lake's surroundings, as well as with a trend of increasing of the water surface observed in the last few years. The number of communities is also associated with a reduction of the surface inhabited by macrophytes and it decreased from 33.9 ha in 2008 to 25.3 ha in 2012 (Fig. 6). An increase of the participation of rush communities in creation of phytolittoral zone also provides enrichment in the nutrients processes in the reservoir (Sender, 2007). Communities of evergreen stoneworts, often treated as an indicator of clean, oligo- and mesotrophic lakes were subsided (Pełechaty, 2006).

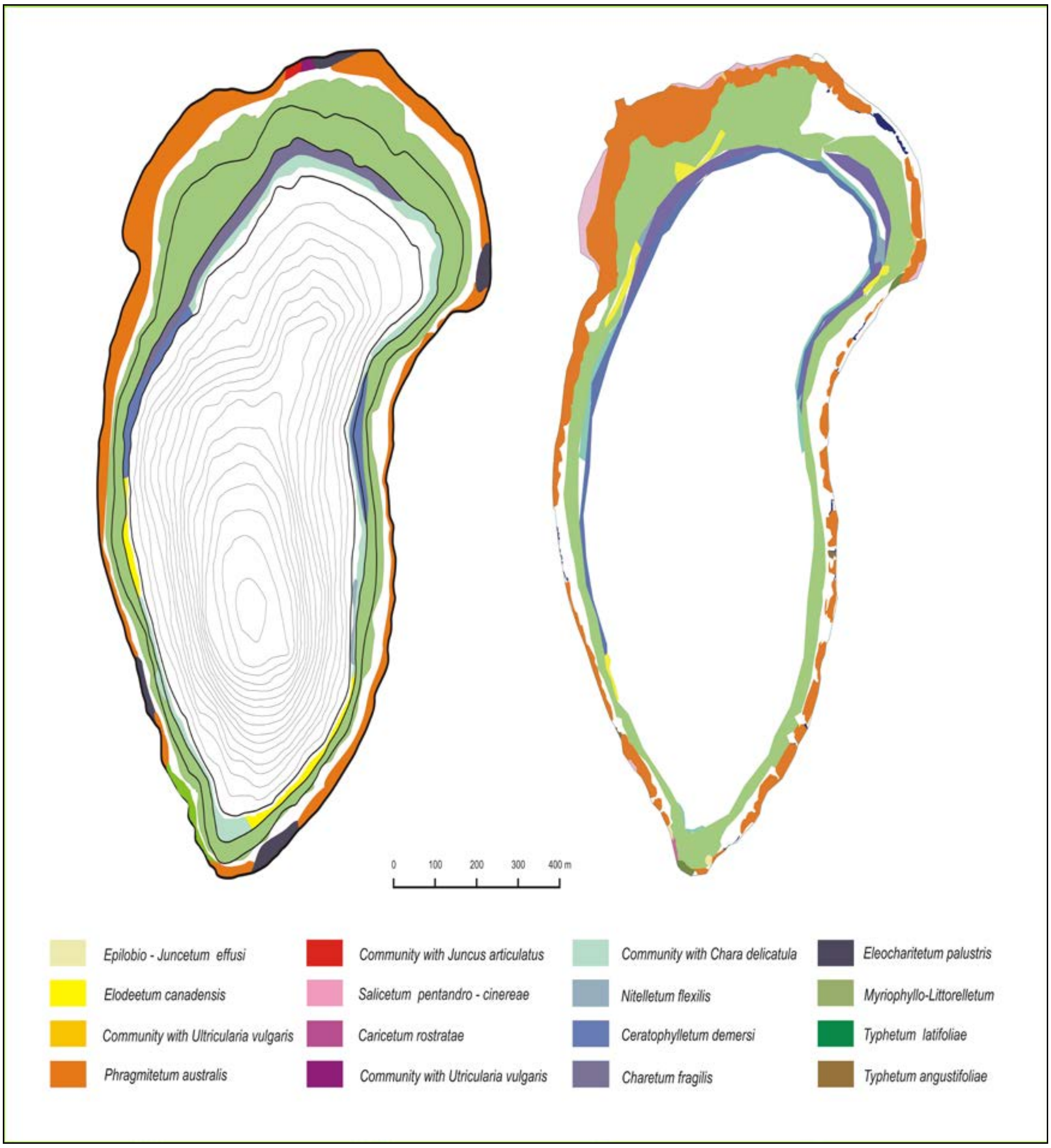

Figure 6: Phytolittoral of the Piaseczno Lake in 2008 and 2012.

CONCLUSIONS

The Piaseczno Lake still harbour very high natural values, but the reduction in the number of macrophyte communities that occurred especially in 2008, was a consequence of the fast growing recreational infrastructure.

From 1976 to 2010, the area of recreation infrastructure in the study area has increased over 3.5 times and the built-up area over five times, as well as roads over twice, while the surface of wetlands and peat bogs significantly decreased - over 11 times.

Long-term changes in the macrophyte communities’ structure showed that the number of communities has varied in each year, probably as a consequence of changes in land-use. The analysis of the surface changes inhabited by macrophytes only over four years has decreased significantly, by about 25%. However, the share of the rush communities increased.

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HIGH MORPHOLOGICAL VARIABILITY OF *GERRIS ARGENTATUS* SCHUMMEL 1832 (GERRIDAE) AND PROBABLY EUROPE'S SMALLEST GERRIDS, IN THE DANUBE DELTA (ROMANIA)

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DOI: 10.2478/trser-2013-0023

KEYWORDS: Heteroptera, *Gerris argentatus*, wing polymorphism, Danube Delta.

ABSTRACT

On a field trip to the Busurca Canal, near Sulina, in the Danube Delta, an apterous *Gerris argentatus* male was captured. It measured only 5.1 mm from the tip of the head to the end of the last abdominal segment, and was the smallest adult pond skater recorded in Europe. This specimen was also the first to be describe as an apterous form of *G. argentatus*, the species previously being known as either macropterous or micropterous. A large macropterous male, measuring 6.77 mm, was also collected in the same habitat, its size exceeding the known measurement for the species. Five *G. argentatus* females were taken from two sites on the Busurca Canal, each one measuring under the 7.5 mm length documented as a minimum for females of the species, with the smallest sample being only 6.59 mm in length.

ZUSAMMENFASSUNG: Die große morphologische Variabilität bei *Gerris argentatus* Schummel 1832 (Gerridae), die wahrscheinlich kleinste Gerriden-Art Europas im Donau-Delta (Rumänien).

Ein flügelloses Männchen von *Gerris argentatus* von lediglich 5,1 mm von der Kopfspitze bis zum letzten abdominalen Segment wurde während wissenschaftlichen Untersuchungen im Bursurca Kanal, nahe der Stadt Sulina im Donau-Delta gesammelt. Dabei handelt es sich um den kleinsten adulten Wasserläufer, der jemals in Europa dokumentiert wurde. Die flügellose Form wird ebenfalls erstmals für *G. argentatus* nachgewiesen, da die Art meist als Macro- oder Micropteere bekannt ist. Im selben Habitat wurde ein großes macropteres Männchen festgestellt, das mit einer Länge von 6,77 mm die für diese Spezies bekannte Größe übertrifft. In zwei der untersuchten Habitate im Bursurca Kanal wurden fünf weibliche Exemplare gesammelt, deren Maße alle weniger als die üblichen, für diese Spezies dokumentierten 7,5 mm betrugen. Das kleinste Exemplar war lediglich 6,59 mm lang.

REZUMAT: Variabilitate morfologică mare la populații de *Gerris argentatus* Schummel 1832 (Gerridae), din Delta Dunării și probabil cel mai mic Gerrid al Europei (România).

Un mascul apter de *Gerris argentatus* a fost capturat într-o campanie de studiu pe Canalul Busurca, în apropiere de Sulina, în Delta Dunării, măsurând doar 5,1 mm de la extremitatea capului până la ultimul segment abdominal, exemplarul fiind cel mai mic Gerrid adult documentat pe continentul european. Forma apteră este, de asemenea, pentru prima dată menționată pentru *G. argentatus*, specie cunoscută ca macropteră sau micropteră. Un mascul de dimensiuni mari (6,77 mm) a fost capturat în același habitat, dimensiunea lui depășindu-le pe cele cunoscute pentru specia în cauză. Cinci femele au fost capturate în două habitate investigate pe Canalul Busurca, toate măsurând mai puțin decât cei 7,5 mm dați ca lungime minimă pentru specie, iar cea mai mică având doar 6,59 mm.

INTRODUCTION

Gerris argentatus Schummel 1832 is one of the most common pond skaters of Europe. Its presence is documented in all continental states of Europe, except Bosnia and Hertzegovina (Poisson, 1957; Aukema and Rieger, 1995; Aukema, 2004; Fent et al., 2011), and it is usually found in stagnant or slow-flowing waters in Romania, especially where hygrophilous vegetation is present (Paina, 1975; Davideanu, 1999). Although samples have been collected at over 1,500 m in the Romanian Carpathians, *G. argentatus* prefers lower altitudes (Davideanu, 1999; Ilie and Olosutean, 2009; Berchi et al., 2011), being regularly found in the Danube Delta (sampled at Caraorman and Crișan - Horváth, 1909; at Gorgova, Sfântu Gheorghe and on the Magearu Canal - Kiss and Davideanu, 1994; near Sulina Beach - Olosutean and Ilie, 2010).

Members of the Gerridae family are semi-aquatic true bugs characterized by a clear sexual dimorphism: the male is smaller than the female because of its reproductive system, with the male attaching on top of the female throughout the reproductive period.

G. argentatus is considered to be the smallest European member of the Gerridae family. Linnavuori (1966) and Nummelin et al. (1998) list species measurements between 6.5 and 8 mm, though without discriminating between males and females. Poisson (1957) and later Davideanu (1999), however, categorize a 5.5 to 6.5 mm length for the male and a 7.5 to 8 mm margin for the female. Andersen (1993) extends the male's length down to 5.2 mm and the female's length up to 8.3, but returns to Poisson and Davideanu's measurements in a later paper (Andersen, 1996).

All authors present the species as macropterous or micropterous. Poisson (1957) describes macropterous individuals as usually larger than micropterous, launching the idea of a coherent relation between wing length and body length in the species.

MATERIAL AND METHODS

Biological material for the study was provided by quantitative samples taken in late August 2012, on the Busurca Canal of the Danube Delta, as part of a larger sampling campaign in the eastern Danube Delta. Each sample was collected over a twenty-five minute period using an entomological net with an 800 cm^2 opening and two mm mesh screening, with the insects later being preserved in 70% ethylic alcohol.

The species was identified using keys from Poisson (1957), Andersen (1993) and Davideanu (1999), while updated taxonomic information was provided by Fauna Europaea (Aukema, 2004).

All photographs and measurements were made using cellSens Entry v. 1.5 ($^{©}$Olympus Corporation), receiving data from an Olympus SZX 16 Stereo Microscope.

RESULTS AND DISCUSSION

G. argentatus individuals were found in only two of the nine habitats investigated in 2012, namely the stations encoded as R5 (around 10 km SSW from the city of Sulina, 45˚04'23.28" N, 29˚35'52.89" E, altitude zero m), and R8 (around six km SSW from the city of Sulina, 45˚06'42.64" N, 29˚35'43.08" E, altitude zero m) (Fig. 1).

At R5, a small, apterous *G. argentatus* male was collected with an individual measurement of 5102.78 µm (Fig. 2) being smaller than the 5.5 mm usually given as the minimum length for the individual was the smallest *G. argentatus* officially recorded, and consequently became the smallest known European pond skater. It is also the first recording of an apterous *G. argentatus* because, all previous authors had labeled the species as either macropterous or micropterous (Poisson, 1957; Andersen, 1993, 1996; Davideanu, 1999).

Figure 1: The location of the only two samples (R5 and R8) containing *G. argentatus*.

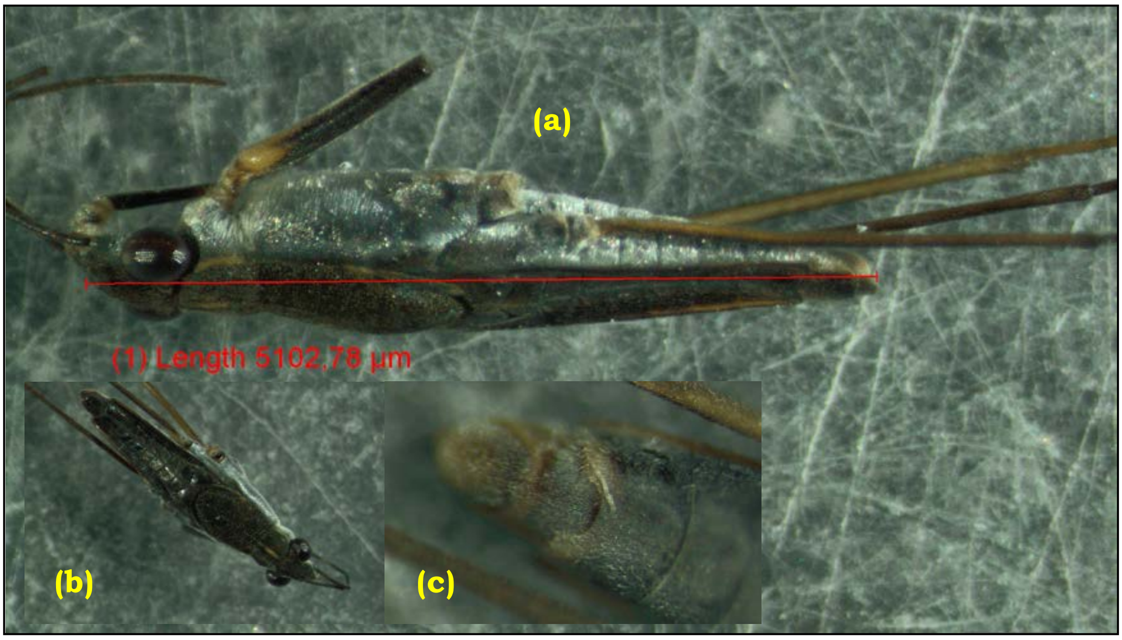

Figure 2: Small apterous *G. argentatus* male: a) lateral view; b) dorsal view; c) genital segments, ventral view.

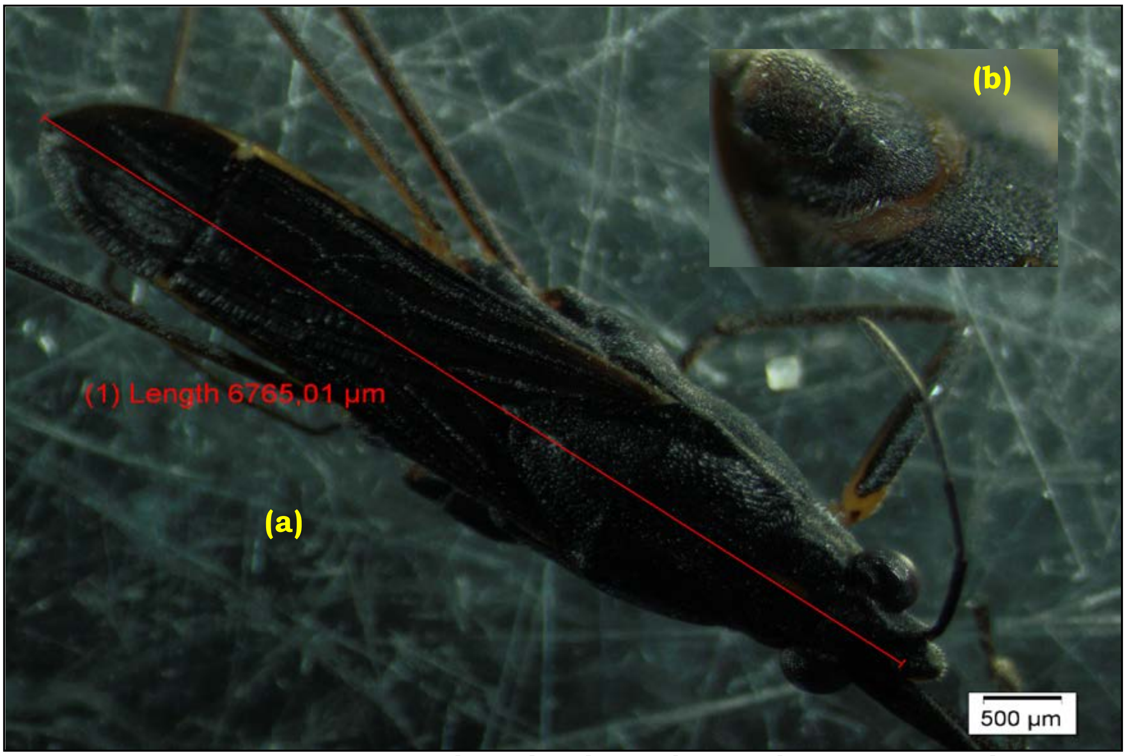

Figure 3: Large *G. argentatus* male: a) dorsal view; b) genital segments, ventral view.

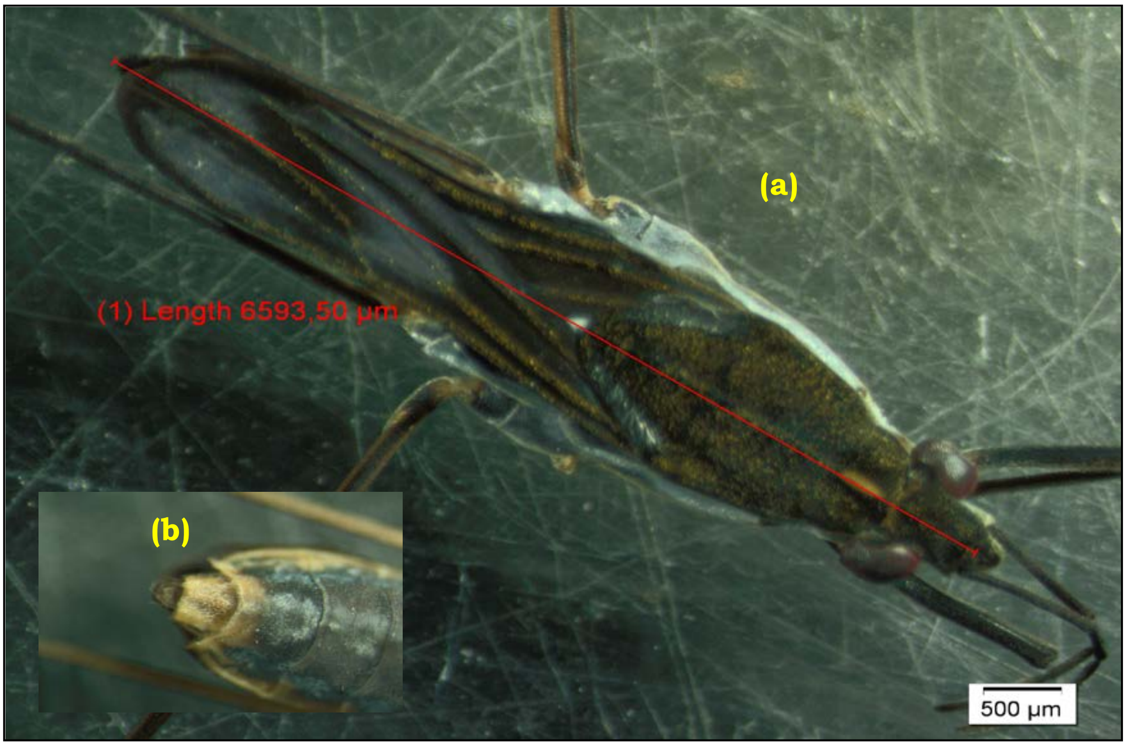

Figure 4: Small *G. argentatus* female: a) dorsal view; b) genital segments, ventral view.

In the same sample there was a large, macropterous male measuring 6765.01 µm, which was larger than any known male specimen, making it the largest *G. argentatus* male recorded at that time (Fig. 3). Two *G. argentatus* females were also retrieved from the sample, with each measuring under the 7.5 mm given as the minimum size (7252.03 µm and 7350.01 µm, respectively).

At R8, a sample of four *G. argentatus* adults was collected, all macropterous: one 5903.92 µm male, and three small females, measuring 7060.64 µm, 7144.46 µm and 6593.50 µm, respectively. The last individual (Fig. 4) was the smallest *G. argentatus* female ever recorded, over 12% shorter than Poisson's (1957) and Davideanu's (1999) minimum length measurements.

R5 and R8 were the only two habitats sampled for *G. argentatus* during the 2012 campaign. European Frogbit (*Hydrocharis morsus-ranae*) was also found at R5 and R8 (Fig. 5), which probably provides suitable shelter for the small *G. argentatus* individuals; the relationship between the two species is currently under study.

Figure 5: *G. argentatus* habitats:
a) typical vegetation, with *Hydrocharis morsus ranae* coverage;
b) gross-plan view of station R8.

The presence of several females that were smaller than the known minimum size of females, from other habitats, together with the small male, could be evidence of a smaller-sized population of *G. argentatus* in this specific area of the Danube Delta. These samples represent the smallest known European population of the Gerridae family. Since micropterous *G. argentatus* are presented as smaller than macropterous ones, there could be a relationship between apterous individuals and even smaller sized pond skaters of this particular species. This possible relationship will be investigated in future campaigns.

On the other hand, the presence of apterous and macropterous individuals and the relatively large size-variability of both males and females (e.g. the 32.5% difference in size between the smallest and the largest male) may lead to the conclusion that the Busurca Canal *G. argentatus* population has a large variability potential. If that is the case it would require, further field studies in order to determine the factors that may affect this variability, along with how important these factors are to the conservation of the species.

CONCLUSIONS

The small population of *G. argentatus*, clearly outside the known measurements for the species, as well as the presence of apterous individuals, is showing that additional studies are necessary in order to establish the morphological range of the species. Large aquatic ecosystems, like the Danube Delta, might be the best locations for extensive studies on other semi-aquatic bugs, which, like *G. argentatus*, might prove to have a higher morphological capacity than what is known today.

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ANALYSIS OF THE MICROSATELLITE VARIATION IN THE COMMON HYBRID BETWEEN RUSSIAN STURGEON (*ACIPENSER GUELDENSTAEDTII* BRANDT AND RATZEBURG, 1833) AND SIBERIAN STURGEON (*ACIPENSER BAERII* BRANDT, 1869) FROM AQUACULTURE

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DOI: 10.2478/trser-2013-0024

KEYWORDS: sturgeon, hybrid, microsatellite, aquaculture, genetic characterization.

ABSTRACT

Sturgeons such as *Acipenser baerii* and *Acipenser gueldenstaedtii* are the most common species farm raised worldwide in aquaculture, because of the dwindling natural sources of caviar and meat. Also, these species can easily participate in the formation of an intraspecific hybrid with a great potential for growth in aquaculture.

Microsatellites are nuclear markers consisting of short repetitive sequence, dispersed across the entire genome with characteristics such as relatively small size and high level of polymorphism. The aims of the present study were to optimize a protocol for microsatellite multiplexing and analysis of genetic diversity in hybrid sturgeons farmed in Romania.

Genomic DNA was isolated from fins, and four pairs of primers were designed to amplify microsatellite loci: LS 19, LS 68, Aox 9, and Aox 45. Amplification of the microsatellite loci was carried out in one 3-Plex reaction for LS 19, LS 68, and Aox 9, and monoplex reaction for Aox 45. For an individual locus we obtained four alleles for Aox 45, eleven alleles for Aox 9, six alleles for LS 68, and eight alleles for LS 19. The results will be applied to test the broodstocks at Romanian hatcheries and to increase the efficiency of breeding.

RÉSUMÉ: Analyse de la variation des microsatellites de l'hybride commun d'aquaculture de l'esturgeon russe (*Acipenser gueldenstaedtii*) et de l'esturgeon sibérien (*Acipenser baerii*).

Les espèces d'esturgeons *Acipenser baerii* et *Acipenser gueldenstaedtii* sont parmi les plus communes dans l'aquaculture de monde entier suite de la diminution des ressources naturelles de caviar et de viande. De surcroît, ces espèces peuvent facilement participer à la formation d'un hybride intraspecifique possèdant un fort potentiel d'élevage.

Les microsatellites sont des marqueurs nucléaires représentés par des séquences répétitives courtes dispersées dans le génome entier et définit par une taille relativement petite ainsi qu'un haut niveau de polymorphisme. Le but de la présente étude est l'optimisation d'un protocole d'amplification de loci microsatellites par PCR multiplex et l'analyse de la diversité génétique des hybrides d'esturgeons d'aquaculture en Roumanie.

L'ADN génomique a été isolée à nageoire. Ainsi, quatre paires d'amorces on été utilisées pour amplifier les microsatellites LS 19, LS 68, Aox 9 et Aox 45. Une réaction d'amplification 3-Plex a été réalisée pour LS 19, LS 68 et Aox 9 ainsi qu'une monoplex pour Aox 45. Pour les loci individuels, nous avons obtenu quatre allèles pour Aox 45, onze allèles pour Aox 9, six allèles pour LS 68 et huit allèles pour LS 19. Le protocole sera appliqué afin de tester la pureté des stocks d'aquaculture des fermes piscicoles de la Roumanie et également pour augmenter l'efficacité de la reproduction.

REZUMAT: Analiza variaţiei microsateliţilor la hibridul comun dintre nisetru (*Acipenser gueldenstaedtii*) şi sturionul siberian (*Acipenser baerii*) din acvacultură.

Sturionii precum *Acipenser baerii* şi *Acipenser gueldenstaedtii* sunt printre cele mai comune specii crescute în acvacultură, în întrega lumea datorită diminuării resurselor naturale de caviar şi carne. Totodată, aceste specii au o capacitate ridicată pentru a participa la formarea unui hibrid interspecific, cu un înalt potenţial pentru creşterea în acvacultură.

Microsateliţii sunt markeri nucleari reprezentaţi de secvenţe repetitive scurte, dispersate în întreg genomul, având caracteristici precum dimensiunile reduse şi un înalt nivel de polimorfism. Scopul prezentului studiu a fost optimizarea unui protocol de PCR multiplex pentru amplificarea de microsateliţi şi analiza diversităţii genetice a hibrizilor de sturioni crescuţi în condiţii de acvacultură în România.

ADN genomic a fost izolat din înotătoare şi patru perechi de primeri au fost desemnaţi pentru amplificarea microsateliţilor LS 19, LS 68, Aox 9 şi Aox 45. Amplificarea a fost realizată într-o reacţie 3-Plex pentru locii LS 19, LS 68 şi Aox 9 şi o reacţie monoplex pentru Aox 45. La nivelul locilor individuali, s-au obţinut four alele pentru Aox 45, unsprezece alele pentru Aox 9, şase alele pentru LS 68 şi opt alele pentru LS 19. Rezultatele vor fi utilizate pentru testarea purităţii stocurilor aparţinând crescătoriilor din România şi pentru creşterea eficienţei reproducerii.

INTRODUCTION

The Danube sturgeon species are among the most valuable species because there is high demand in the global market for both their meat and their roes (caviar). A special measure to develop the aquaculture system for sturgeons in Romania should be considered to reduce the pressure of overfishing and to continue the programs for populating the Danube River with sturgeon spawn. Nowadays, the order of Acipenseriformes counts 25 sturgeon and two paddlefish species, the majority being at the brink of extinction. Because of the commercial value of their roes (caviar), the sturgeon populations all over the world have been overexploited by fishing and poaching. The construction of dams and pollution completed the destructive effect of the anthropic intervention by affecting their habitat and, particularly, their breeding areas. Given their great value, there are many global concerns for sturgeons' protection. For conservation and restoration of sturgeon species, numerous measures have been taken both nationally and internationally. IUCN (International Union for Conservation of Nature) and CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) listed acipenserids in their lists and appendices concerned with conserving threatened species. (Bemis et al., 1997)

The natural range of Siberian sturgeon, *Acipenser baerii,* is very large, the species being present in all hydrographic basins from Siberia, from the river basin of Ob-Irtych in North-West Siberia to the river basin of Kolyma in North-Est Siberia. A particular population of Siberian sturgeon is also present in the hydrographic system of the Baikal Lake. Thus, three subspecies have been distinguished for the Siberian sturgeon: *Acipenser baerii baerii* in the Ob river basin, *Acipenser baerii stenorrhynchus* starting with the Ienisseï River basin as far as the Kolyma River basin and *Acipenser baerii baicalensis* in the Baikal Lake system (Matallanas, 1997).

The farming of this species began in the former USSR in the 1970s and now the farming activities occur in many other countries from all continents. Rearing conditions in farms are most of the time more favorable than the natural conditions where they originate, and puberty occurs considerably earlier, at about six years old for the males and seven years old for the females (Matallanas, 1997).

The species has also been the subject of various hybridizations. One of the species participating in the formation of hybrids with *A. baerii* is *A. gueldenstaedtii* and the new hybrid has a great potential for growth in aquaculture.

Acipenser gueldenstaedtii (Russian sturgeon) is an anadromous species. For breeding, the Russian sturgeons enter the rivers, far away from the mouth, in two migration periods: spring, to reproduce in the same year, and autumn, to reproduce the next spring (Oțel, 2007). *A. gueldenstaedtii*, as well as *Huso huso* or *Acipenser stellatus*, does not migrate every year for reproduction, but at intervals of two to five years. Currently, the species can be found along the Black Sea coast and the Danube River, from the mouth to the Iron Gate Dam, but in the past migrated near Budapest (Oțel, 2007). *A. gueldenstaedtii*, a species with ~250 chromosomes, is considered to be, by the majority of the authors, a tetraploid species with an octaploid ancestor from which some microsatellite loci with octasomic profile are still maintained.

Microsatellites are nuclear markers consisting of a short repetitive sequence (2-9 bp), dispersed in the entire genome with characteristics such as relatively small size, easiness of amplification, codominant inheritance and high level of polymorphism. Such repetitions are highly variable, enabling that location to be tagged or used as a marker. Their advantages (high level of polymorphism, a higher power of discrimination comparative to other genetic markers, codominant mendelian inheritance) make microsatellite markers suitable for genetic research in wild and aquaculture stocks. Specific primers developed to amplify the microsatellite loci from one species can often lead to similar loci amplification of closely related species (Estoup and Angers, 1998), thus representing an advantage especially in small population analysis. The sturgeon studies based on analysis of microsatellite markers were started on North American species (McQuown et al., 2000; King et al., 2001; Pyatskowit et al., 2001; Henderson-Arzapalo and King, 2002; Welsh et al., 2003; Welsh and May, 2006; Fopp-Bayat, 2010) and now, many species have been studied in terms of genetic diversity, despite the difficulties of collecting biological samples reflected in the low number of individuals analyzed. For the European sturgeon species, genetic diversity studies were more limited. Initially, a number of species-specific microsatellites for *Acipenser naccarii* were developed in efforts to restore the species in Pad River (Italy), but loci analysis proved to be difficult due to the nature of this tetraploid species (Zane et al., 2002; Forlani et al., 2008). For Ponto-Caspian species such analyses were carried out less, the only significant studies using microsatellite markers were those concerning the population of *A. stellatus* from the Caspian Sea (Norouzi and Pourkazemi, 2009) and populations of *A. gueldenstaedtii* from the north-west of the Black Sea, the Northern part of the Caspian Sea and the Azov Sea (Timoshkina et al., 2009).

The major aim of the present study was the application of microsatellite DNA for analysis of genetic diversity in common hybrids between *A. gueldenstaedtii* and *A. baerii*, farmed at a hatchery in Romania. The results will be applied to test the purity of hatchery broodstocks at Romanian fish farms and to increase the efficiency of selective breeding and performance testing programs.

MATERIAL AND METHODS

Sampling and DNA extraction

Microsatellite loci were examined by using samples harvested without endangering the life of the individuals. Fin clips were collected from 25 hybrids (*A. baerii* x *A. gueldenstaedtii*) from a Romanian Fish Farm. Genomic DNA was extracted from fin tissue by phenol-chloroform method with minor modifications (Taggart et al., 1992).

Microsatellite amplification

For the microsatellite analysis we used four primer pairs to cross-amplify the following loci: LS 19, LS 68, Aox 9 and Aox 45. These primers were initially designed in North-American sturgeons (Tab. 1). Initially, the PCR conditions were optimized by varying the annealing temperature between 50-61ºC on a gradient thermocycler IQCycler (BioRad). For the microsatellite loci detection we used the forward primers labeled with four different fluorescent dyes: PET, VIC, 6-FAM, NED (Tab. 1). Amplification of the microsatellite loci was done by one 3-Plex reaction for Aox 9, LS 19 and LS 68 and a monoplex reaction for Aox 45.

Table 1: Primer sequences for the microsatellites loci.

Primer	**Sequence**	**Species**
LS 19 F LS 19 R	6-FAM-CATCTTAGCCGTCTGGGTAC CAGGTCCCTAATACAATGGC	*Acipenser fulvescens*
LS 68 F LS 68 R	NED-TTATTGCATGGTGTAGCTAAAC AGCCCAACACAGACAATATC	*Acipenser fulvescens*
Aox 9 F Aox 9 R	VIC-GATATTGGAGCTGTGCATTG ACATTGTTTGGTAGGCCAGC	*Acipenser oxyrhinchus*
Aox 45 F Aox 45 R	PET-TTGTTCAATAGTTTCCAACGC TGTGCTCCTGCTTTTACTGTC	*Acipenser oxyrhinchus*

PCRs were done in 25 μL final volume with PCR Buffer, $MgCl_2$, 200 μM of each nucleotide, DNA template, 0.3 μL of each primer, two units of AmpliTaq Gold DNA Polymerase and nuclease free water. The 3-Plex reaction was carried out on GeneAmp 9700 PCR System (AppliedBiosystems) under the following conditions: initial denaturing step at 95ºC for ten minutes, 35 cycles of denaturing for 30 seconds, 54ºC annealing for 30 seconds, 72ºC extension for 60 seconds; and a final extension at 72ºC for 60 minutes. For the monoplex reaction we followed the same conditions, but the annealing step was at 47ºC. The amplified fragments were loaded with the GeneScan-500 LIZ Size Standard into ABI Prism 310 DNA Genetic Analyzer.

Data analysis

The results were analyzed with the GeneScan 3.1.2. and Genotyper 2.5.2. Softwares (AppliedBiosystems). The statistical analysis was done using the GENETIX software (Belkhir et al., 2002).

RESULTS AND DISCUSSION

Due to certain characteristics, for instance, polyploidy, the sturgeon species hybridize more easily than other fish species (Birstein et al., 1997). It is a well-known fact that sturgeons hybridize in natural conditions leading, sometimes, to fertile intergeneric or interspecific hybrids. In aquaculture the interspecific hybridization has been used to increase the growth rate and disease resistance, improve tolerance to environmental extremes and improve a variety of other traits that make aquatic animal production more profitable.

The main limitation in microsatellite analysis in sturgeons is related to the complexity of their genome. Some authors consider that the sturgeon species with ~120 chromosomes such as *A. stellatus*, *H. huso* or *A. ruthenus* are tetraploids (Birstein et al., 1997), while these with ~250 chromosomes (e.g. *A. gueldenstaedtii*) are octaploids. Other authors consider that they are functional diploids, respectively tetraploids (Fontana et al., 1998; Tagliavini et al., 1999). The Siberian sturgeon belongs to the group of sturgeons with a chromosome number of around 250 (Birstein et al., 1997). The analysis of the genetic diversity highlights the relationships between individuals and may be a useful tool in directing specific crossbreeding. Measuring genetic diversity in wild fish populations or aquaculture stocks is essential for interpretation, understanding and effectively managing these populations or stocks.

Table 2: Characteristics of the microsatellite loci.

Locus	**Size (bp)**	**Number of alleles**	**Pattern**
LS 19	118-145	8	Polysomic
LS 68	200-252	6	Polysomic
Aox 9	194-246	11	Polysomic
Aox 45	132-236	4	Polysomic

In our study, we have successfully amplified all four microsatellite loci for all the hybrids. The size of the alleles and the pattern of each locus are presented in table number 2.

Genotypes were determined for all individuals. The number of allele peaks depends on the level of ploidy of the analyzed species and on whether the individual tested is heterozygote or homozygote. The size of the alleles at individual loci varied between 118 and 252 bp. A medium level of polymorphism was observed for the population of hybrids studied. Four to 11 alleles where observed with an average of 7.25 alleles per locus. The most polymorphic locus is Aox 9. Aox 45 or LS 68 presents a lower polymorphism in the population. All four loci presented a polysomic profile. The size of the alleles and the pattern of each locus are shown in table number 3.

Table 3: Allele frequencies for the microsatellite loci.

LS 19/ Allele	Frequencies	**LS 68**/ Allele	Frequencies	**Aox 9**/ Allele	Frequencies	**Aox 45**/ Allele	Frequencies
118	0.0714	**128**	0.1111	**194**	0.0870	**132**	0.2500
124	0.0238	**148**	0.2500	**196**	0.0435	**153**	0.2500
127	0.1905	**200**	0.1111	**208**	0.1522	**233**	0.2500
130	0.1905	**228**	0.1389	**214**	0.0652	**236**	0.2500
133	0.1905	**232**	0.1667	**218**	0.0870		
136	0.0476	**248**	0.1111	**222**	0.1739		
142	0.1429	**252**	0.1111	**226**	0.0652		
145	0.1429			**228**	0.0217		
				240	0.1087		
				242	0.0652		
				246	0.1304		

Examples of electrophoregrams for the hybrid specific loci are shown in figures 1-4.

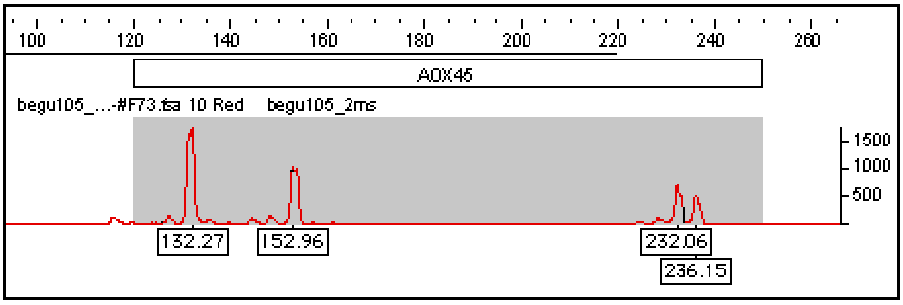

Figure 1: Genotyper software analysis of PCR amplification product for Aox 45 microsatellite locus.

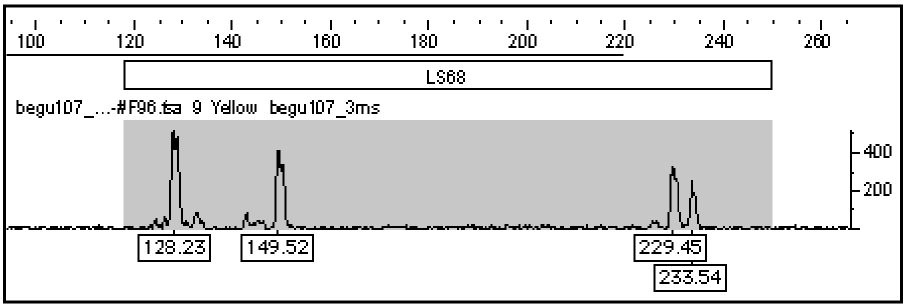

Figure 2: Genotyper software analysis of PCR amplification product for LS 68 microsatellite locus.

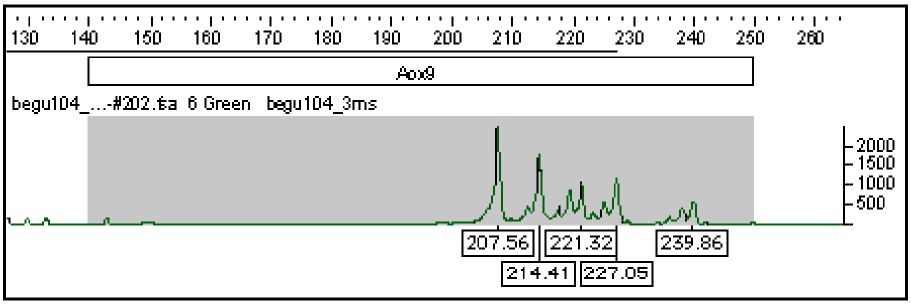

Figure 3: Genotyper software analysis of PCR amplification product for Aox 9 microsatellite locus.

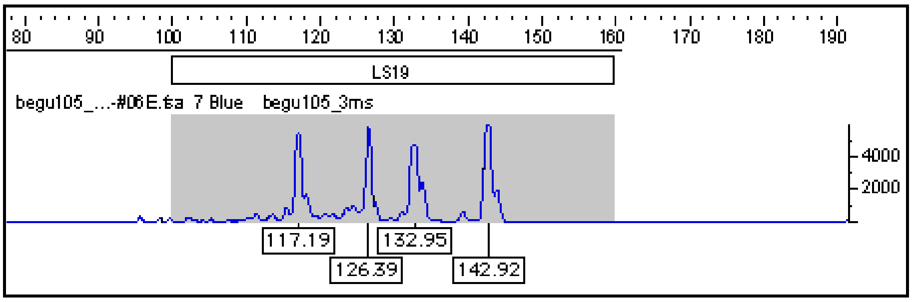

Figure 4: Genotyper software analysis of PCR amplification product for LS 19 microsatellite locus.

CONCLUSIONS

The present study showed that sturgeons bred in aquaculture have a low genetic diversity due to an inbreeding effect. Also, from a statistical point of view, it is impossible to analyze polyploid genotypic data together with diploid genotypic data. Therefore, for an estimation of the genetic diversity in hybrids between *A. baerii* and *A. gueldestaedtii* we propose to isolate and to analyze only the disomic loci in the future.

The need for molecular studies will be increased in the future, thus allowing characterization of stocks and correct identification of individuals.

ACKNOWLEDGEMENTS

This work was supported by the PN-II-PT-PCCA Project 116/2012 “Genetic evaluation and monitoring of molecular and biotechnological factors that influence productive performance of Danube sturgeon species bred in intensive recirculating systems”.

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GENETIC DIVERSITY OF BROWN TROUT POPULATIONS USING MITOCHONDRIAL MARKERS IN RELATIVELY SIMILAR GEOGRAPHICAL AND ECOLOGICAL CONDITIONS – A CARPATHIAN CASE STUDY

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DOI: 10.2478/trser-2013-0025

KEYWORDS: Făgăraş Mountains, brown trout, D-loop, genetic diversity.

ABSTRACT

Among the species of salmonids that exist in Romania, the most common is the brown trout (*Salmo trutta*, morpha *fario*, Linnaeus, 1758), with a high commercial potential, being used for aquaculture or fishing. Unfortunately, its natural habitat is disrupted by human activities and in order to avoid local extinction of *Salmo trutta fario* repopulation strategies are being applied. The repopulation activities must be carefully designed and conducted taking into consideration that there is a high risk of hybridization. Our study aims to analyze the genetic diversity of three Romanian brown trout populations found in rivers in the Făgăraş Mountains using mitochondrial markers.

Standard DNA extraction protocol with phenol-chloroform was applied to the biological material represented by small fin fragments sampled from 80 individuals of brown trout, followed by PCR amplification of D-loop control region and Sanger sequencing. Alignment and editing of all the sequences obtained were carried out with Bioedit, the phylogenetic tree construction was performed by Neighbour Joining method implemented in MEGA v5, and intra- and interpopulational diversity was evaluated with DNAsp v5 and MEGA v5.

For the three brown trout populations, 13 haplotypes were identified for N1, 11 for N2 and 10 for N4, with a haplotype diversity greater than 0.8. The phylogenetic tree topology showed that individuals chosen for this study were placed in the Danubian clade as the other Danubian sequences selected from GenBank.

The study proved that the three *Salmo trutta fario* populations analyzed were genetically distinct and that in Romania there are still are pure Danubian brown trout populations.

RÉSUMÉ: La diversité génétique des populations de truites brunes à l’aide de marqueurs mitochondriaux dans des conditions géographiques et écologiques relativement similaires - une étude de cas des Carpates.

Parmi les espèces de salmonidés qui existent en Roumanie, la plus commune est la truite brune (*Salmo trutta fario*, Linnaeus, 1758) qui possède un grand potentiel commercial, étant utilisées pour l’aquaculture ou la pêche. Malheureusement, son habitat naturel est perturbé par des activités humaines. Dans le but d’éviter l’extinction de la truite, des stratégies de repeuplement sont appliquées. Les activités de repeuplement doivent être soigneusement conçues et réalisées en tenant compte d’un grand risque d’hybridation. Le but de notre étude est l’analyse la diversité génétique de trois populations de truites brunes roumaines qui se trouvent dans les rivières des Monts Fagărăş à l’aide de la région de contrôle de l’ADN mitochondrial. Le protocole standard d’extraction de l’ADN, au phénol-chloroforme, a été appliqué sur les échantillons biologiques représentés par de petits fragments de nageoire appartenant à 80 individus de truite brune, suivie par l’amplification de la région de contrôle D-loop par PCR ainsi que le séquençage par la méthode Sanger. L’alignement de toutes les séquences obtenues a été réalisé avec Bioedit, la construction de l’arbre phylogénétique a été réalisée par la méthode de Neighbor Joining (logiciel MEGA v5) et la diversité intra- et inter-populationnel a été évaluée avec DNAsp v.5 et MEGAv.5.

Pour les trois populations de truites brunes, 13 haplotypes ont été identifiés pour N1, 11 pour N2, et 10 pour N4, avec une diversité d’haplotypes supérieur à 0,8. La topologie de l’arbre phylogénétique a montré que les truites choisies pour cette étude ont été placées dans le clade danubien, comme les autres séquences du Danube choisis dans GenBank.

Cette étude a prouvé que les trois populations de *Salmo trutta fario* analysées sont génétiquement distinctes et qu’en Roumanie, il existe toujours des populations de truite brune pures danubiennes.

REZUMAT: Analiza diversităţii genetice cu ajutorul markerilor mitocondriali, a unor populaţii de păstrav comun aflate în condiţii geografice şi ecologice relativ similare în Munţii Carpaţi.

Păstrăvul comun reprezintă una dintre speciile de Salmonidae din România, cu o importanţă comercială deosebită, utilizat în domeniul acvaculturii şi pentru pescuit sportiv. Din nefericire, influenţa antropică se resimte şi în habitatul lui *Salmo trutta fario*, iar pentru a contracara influenţele negative, se încearcă aplicarea de strategii de repopulare. Activitățile de repopulare trebuie să fie realizate și desfăşurate cu atenţie, luând în considerare faptul că există un risc ridicat de hibridizare. În cadrul acestui studiu, am avut în vedere analiza diversităţii genetice a trei populaţii de păstrăv comun din regiunea nordică a Munţilor Făgăraş, folosind markeri mitocondriali.

Extracţia de ADN a fost realizată din mici fragmente de înotătoare, provenite de la 80 de indivizi, urmând protocolul standard de extracţie cu fenol-cloroform, urmată de amplificare prin PCR a regiunii mitocondriale D-loop şi secvenţiere după metoda Sanger. Alinierea şi editarea secvenţelor obţinute a fost realizată cu programul BioEdit, iar arborele filogenetic a fost construit prin metoda Neighbour Joining din cadrul programului MEGA v.5. Diversitatea inter- şi intrapopulaţională a fost evaluată cu programele DNAsp v.5 şi MEGA v.5.

Pentru cele trei populaţii analizate, am observat că există 13 haplotipuri în cadrul populaţiei N1, 11 în populaţia N2 şi 10 în populaţia N4, cu o diversitate haplotipică mai mare de 0,8. Topologia arborelui filogenetic a sugerat că indivizii analizaţi au fost grupaţi în clada danubiană, alături de alte secvenţe aparţinând liniei danubiene utilizate la construcţia dendrogramei.

Acest studiu a demonstrat ca cele trei populații de *Salmo trutta fario* analizate sunt distincte din punct de vedere genetic şi că în România încă există populații pure de păstrăv comun danubian.

INTRODUCTION

Among the Salmonidae species that exist in the Romanian national territory, *Salmo trutta fario* Linnaeus, 1758, *Salmo trutta lacustris* Linnaeus 1758, *Salmo trutta labrax* Pallas 1811, *Salmo gairdneri irideus* Gibbons 1855, *Salvelinus fontinalis fontinalis* (Mitchil) 1815, *Hucho hucho* (Linnaeus) 1758, (Bănărescu, 1964; Bănărescu and Bănăduc, 2007), the most common and also the most important from economic point of view is the brown trout (*Salmo trutta*, morpha *fario*, Linnaeus, 1758). Representatives of this species are found in alpine lakes from Făgăraş, Parâng and Retezat Mountains, and almost all mountain springs from Romania. *Salmo trutta fario* is known as a rapacious fish that prefers to ambush its prey in various locations such as rocks, waterfalls or fallen trees (Georgescu et al., 2011), eating insects and larvae. It reaches 25-50 cm in length and 0.2-5 kg in weight, and has its reproduction period in late autumn (Bănărescu, 1964).

The brown trout has great commercial potential, its meat being well appreciated, and often it is used for rising in aquaculture conditions. Particularly, in Romania, a great interest in fish farming of brown trout has been manifested since 1890 (Georgescu et al., 2011).

Because of its wide geographical distribution, which runs from Norway to eastern Russia and from Iceland to the tributaries of the Aral Sea, the brown trout shows great diversity concerning phenotypic characteristics (Bernatchez et al., 1992). By using molecular markers, especially the mitochondrial control region D-loop, it was shown that in Europe there are five main lineages of *Salmo trutta*: Atlantic, Danubian, Marmoratus, Mediterranean and Adriatic (Bernatchez et al., 2001). The D-loop control region is a non-coding region, located between the genes for $tRNA^{Pro}$ and $tRNA^{Phe}$, that contains the initiation signals for mitochondrial genome replication (Shadel et al., 1997). Other studies were focused on using RFLP technique to study the genetic differentiation of Salmonids (Maric et al., 2006; Dudu et al., 2010; Apostolidis et al., 1996) or on 16S and 12S rRNA genes sequences to study the phylogenetic classification of Romanian salmonid species (Dudu et al., 2010). The mitochondrial genome has some characteristics that make it useful in phylogenetic and populational studies: fast evolution rate, higher mutation rate than the nuclear genome, maternal heritage - useful for hybrid identification studies - and relatively compact structure.

Unfortunately, the brown trout’s natural habitat is disrupted by a series of human activities such as excessive fishing, river dams and hydropower plants construction on main mountain rivers, garbage disposal and ballast exploitation. In order to avoid endangerment or even local extinction of *Salmo trutta fario*, repopulation strategies are being applied, frequently with individuals that come from aquaculture. Uncontrolled population restocking with non-native brown trout individuals often used in aquaculture might lead to hybrid formation, affecting the genetic diversity of natural *Salmo trutta* populations (Guyomard, 1989). Furthermore, the classification of resulting hybrids is difficult to achieve based exclusively on morphology, and a genetic analysis is recommended (Boaru, 2008).

Although in Romania *Salmo trutta fario*'s morphological characteristics are widely described (Bănărescu, 1964; Bănărescu and Bănăduc, 2007), studies concerning the genetic diversity of Romanian brown trout are rare. Until now, there have been no complete studies about its genetic characteristics and this could affect the future potential repopulation programmes that in the past have not taken a systematic approach in this respect.

Our preliminary study aims to analyze the genetic diversity of three Romanian brown trout populations from rivers in the Făgăraş Mountains area using the D-loop control region as a mitochondrial marker.

MATERIAL AND METHODS

DNA extraction

The biological samples were represented by small anal fin fragments from 80 individuals belonging to three natural populations from the Northern side of the Făgăraş Mountains, tagged as N1, N2 and N4. The DNA extraction was done using standard phenol-chloroform protocol (Taggart, 1991) with minor modifications and elution in purified water.

PCR optimization and amplification

The amplification of the entire D-loop region was done using the primers forward 5'CCCAAAGCTAAAATTCTAAAT3' and reverse 5'CCCAAAGCTAAAATTCTAAAT3'. Initially, we optimized the PCR reaction conditions by varying the annealing temperature between 46 and 60°C. The PCR program that we use for amplification of 1012 bp mitochondrial region of D-loop was as follows: denaturation at 95°C for ten minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C and extension at 72°C for one minute and final extension at 72°C for ten minutes. The PCR reactions were carried out on GeneAmp 9700 PCR System (AppliedBiosystems) with a final volume of 25μl that contained 1X PCR Buffer, 1.5 mM of MgCl2, 0.8 mM of dNTPs, two pmol/μl of each primer, one unit of AmpliTaq Gold DNA polymerase, nuclease free water and 50 ng of DNA template. The PCR products were analyzed by electrophoresis on 2% agarose gel in TAE 1X.

PCR products purification and sequencing

The purification of PCR products was carried out with Wizard SVGel and PCR Clean-Up System (Promega), followed by Sanger sequencing using Big Dye Terminator v3.1 kit (Applied Biosystems) and the sequencing products were loaded on the 3130 Genetic Analyzer (Applied Biosystems) after a previous purification with BigDye XTerminator® Purification Kit (Applied Biosystems).

Sequence alignment

The sequences were edited, compared and aligned to other brown trout D-loop sequences downloaded from Gene Bank (accession number GQ284837) and truncated to 903bp by using Bioedit (Hall, 1999).

Data analysis

In the phylogenetic analysis, besides our sequences, we used control region sequences from GenBank database that belong to four different lineages: Atlantic, Danubian, Mediterranean and Adriatic (Tab. 1). The phylogenetic tree construction was performed by Neighbor Joining method implemented in MEGA v5 and *Salmo salar* (GenBank accession number U12143) was selected as outgroup. The intra- and interpopulational diversity was evaluated with DNAsp v5 (Librado et al., 2009) and MEGAv5 (Tamura et al., 2011).

Table 1: The accesion number and lineage of each external control region sequence used for phylogenetic analysis.

Lineage	Accesion number (GenBank)
Adriatic	AY836330
	DQ381566
	AY836331
	DQ381567
Atlantic	AF273086
	AF274574
	AY185578
	AF273087
	AY185577
Mediterranean	AY836361
	AY836364
	AY836362
	AY836363
Danubian	AY185571
	GQ284837

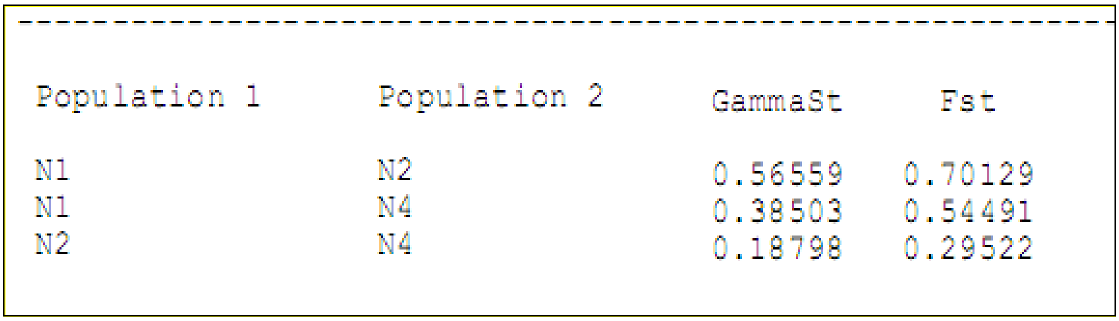
RESULTS AND DISCUSSION

All 80 complete D-loop sequences of 1012 bp obtained from *Salmo trutta fario* were aligned with a similar sequence from GenBank and after truncation to 903 bp they were used for analysis with DNAsp, v5. We aimed to explore the inter- and intrapopulational diversity and discovered that the number of polymorphic sites was as follows: 9 for the N1 population, 24 for the N2 population and 14 for the N4 population (Fig. 1), most of them being transition type polymorphisms. The haplotype diversity for each of all three populations was relatively high, with values > 0.8 (Tab. 2). These values suggest that there is a significant level of genetic variation within all three analyzed populations. For the three *Salmo trutta fario* populations, 13 haplotypes were identified for N1, 11 for N2 and 10 for N4 (Tab. 2).

Table 2: The analysis of three brown trout populations concerning the number of polymorphic sites, number of haplotypes and haplotype diversity by using DNAsp v5.

Population	Number of polymorphic/indel/missing sites	Number of haplotypes	Hd (haplotype diversty)
N1	9	13	0.865
N2	24	11	0.826
N4	14	10	0.852

The genetic differentiation degree between the three populations was also evaluated with DNAsp and by Wrigth statistics (Fig. 1). We observed that the highest values (> 0.25) both for Fst and Gammast indices were obtained between the N1 and N2 populations (Fst = 0.7, Gammast = 0.56) and the lowest between the N2 and N4 brown trout populations. The values of the analyzed statistical indices reflect that the populations are genetically distinct and the highest degree of genetic differentiation is between the N1 and N2 populations, while the lowest is between N2 and N4.



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Population 1        Population 2        GammaSt     Fst

N1                  N2                  0.56559   0.70129
N1                  N4                  0.38503   0.54491
N2                  N4                  0.18798   0.29522
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Figure 1: The values of the Wright statistical indices for the three analyzed brown trout populations (N1, N2 and N4) evaluated with DNAsp.

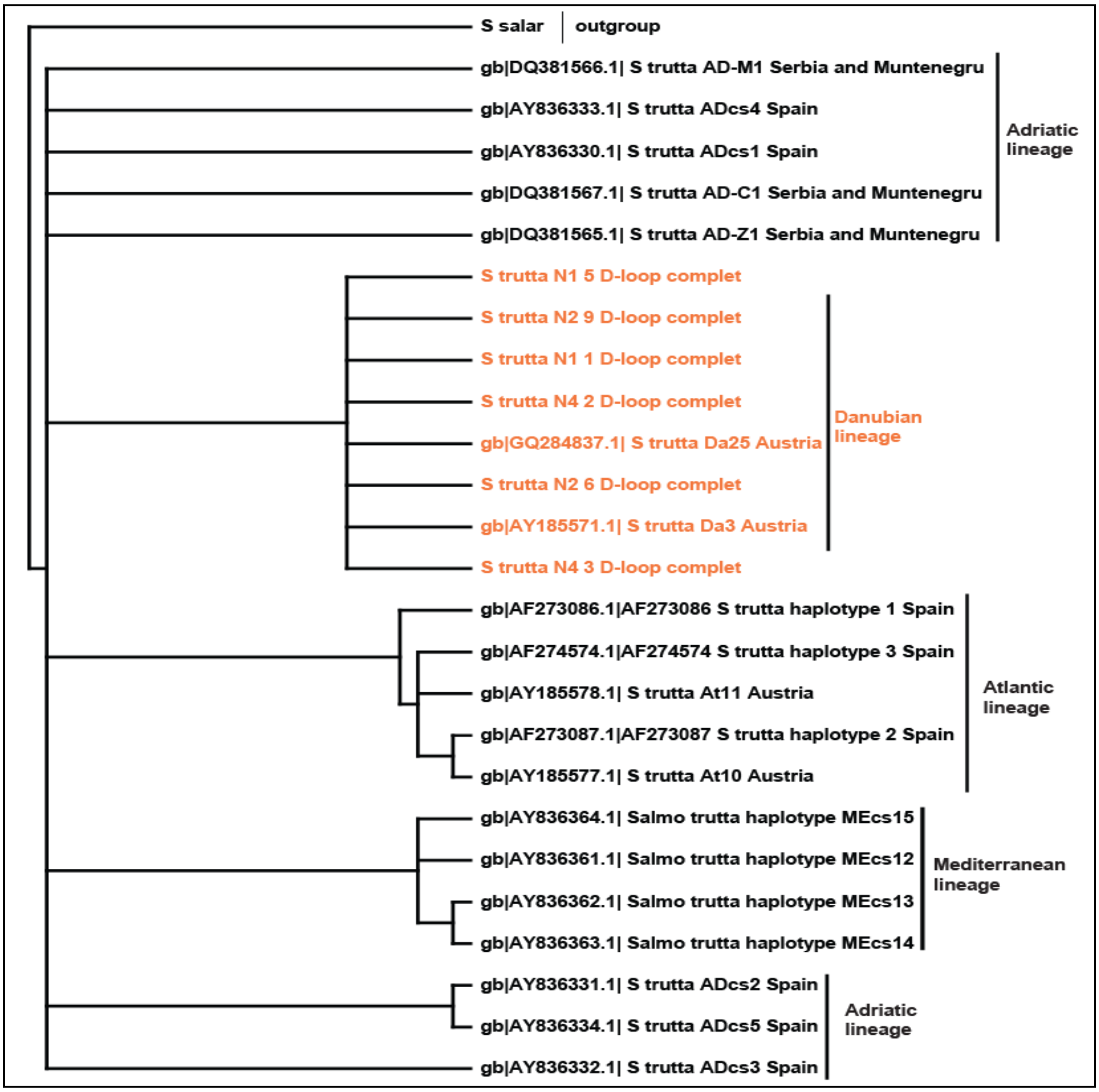

Figure 2: Phylogenetic tree obtained with MEGAv5 (NJ method, bootstrap value 1000 replications) illustrating the relationships between the evolutive lineages of brown trout.

The phylogenetic tree resulting from analysis with MEGA v.5 contained, besides our *Salmo trutta fario* sequences, complete D-loop sequences from other European lineages in order to observe the affiliation of analyzed Romanian individuals. The dendrogram topology showed a classification of analyzed sequences in distinct monophiletic groups corresponding to each evolutive lineage, and that the representatives of brown trout from Romania analyzed in this study were placed in the Danubian clade similar to other sequences selected from GenBank and belonging to Danubian lineage (Fig. 2).

In Romania, we expected to find individuals from the Danubian lineage and this was found to be the case, meaning that either restocking programs have not been done yet or, if they have, the individuals involved were selected properly.

The genetic diversity of Romanian brown trout concerns us due to ongoing construction of hydropower plants on several mountain rivers in the northern side of the Făgăraş Mountains. Also, in the area, there are several trout farms from which it is possible for non-indigenous individuals to escape, leading to hybrid formation. All of these anthropic activities might severely degrade the aquatic ecosystems.

Our next steps are to evaluate the genetic diversity in a higher number of populations of *Salmo trutta fario* from other Romanian mountain regions in order to ensure a better view upon the actual state of brown trout in our country.

CONCLUSIONS

To conclude, it was proven that the three analyzed *Salmo trutta fario* populations were genetically distinct and that in Romania there still are pure Danubian brown trout populations. This study is a first step in the molecular characterization of *Salmo trutta fario* from Romania using mitochondrial DNA markers and could provide precious information in future management strategies.

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Transylv. Rev. Syst. Ecol. Res. 15.2 (2013), "The Wetlands Diversity"

REPRODUCTIVE ECOLOGY OF MANGROVE FLORA: CONSERVATION AND MANAGEMENT

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DOI: 10.2478/trser-2013-0026

KEYWORDS: mangroves, vivipary, crypto-vivipary, reproductive ecology, conservation, management.

ABSTRACT

Mangroves are dynamic and unique inter-tidal ecosystems, common in tropical and subtropical coastal environments. They are among the world's most productive ecosystems and are important in protecting coasts from erosion by fierce tides, in promoting the diversity of marine organisms and fisheries by contributing a quantity of food and providing favourable habitats for animals. These economic uses of mangroves indicate that they play an important role in the lives and economies in the coastal regions of different countries. Mangrove forests are under immense threat worldwide due to their multiple economic uses and alterations of freshwater inflows by various upstream activities in catchment areas. Mangrove plants with unique adaptations play a crucial role in sustaining life in mangrove forests. Their reproductive biology is central to understanding the structural and functional components of mangrove forests.

The success of sexual reproduction and subsequent population expansion in mangrove plants is linked to flowering timings, pollinators and tidal currents. Viviparous and crypto-viviparous plants are true mangroves while non-viviparous ones are mangrove associates. The dispersal propagule is seedling in viviparous and non-viviparous plants while it is seed in non-viviparous plants. In this study, viviparous and crypto-viviparous species were included for study. These species are self-compatible, self-pollinating and also cross-pollinating; such a breeding system is a requirement for the success of sexual reproduction and subsequent build up and expansion of population. They are entomophilous in the study region. The viviparous plants include *Ceriops tagal, C. decandra, Rhizophora apiculata, R. mucronata, Bruguiera gymnorrhiza* and *B. cylindrica.* The non-viviparous plants include *Avicennia alba, A. marina, A. officinalis, Aegiceras corniculatum* and *Aegialitis rotundifolia.* Sexual reproduction and regeneration events are annual in these plants and are dependent on local insects, tidal currents and nutrient content in estuarine environment.

In recent times, erratic and insufficient rainfall together with industrial pollutants released into rivers is causing negative effects on the growth, development and regeneration of mangrove flora. In effect, there is a gradual decrease in mangrove cover. Added to this is continuous exploitation of mangrove plants for fuel wood, creation of shelters for cattle and changes for industrial establishments and aquaculture development in estuarine regions. As a consequence, the existing mangrove cover is struggling to survive and also not in a position to support local needs and provide livelihood opportunities through fishery resources. Further, reduced mangrove cover is showing catastrophic effects on fishing communities who live along the shore line during the period of cyclonic surges and tsunami events.

ZUSAMMENFASSUNG: Vermehrungsökologie der Mangrovenflora: Erhaltung und Management.

Mangroven sind dynamische und einzigartige Gezeiten-Ökosysteme, die in tropischen und subtropischen Küsten-Gebieten häufig vorkommen. Sie gehören zu den weltweit produktivsten Ökosystemen und sind durch ihren fördernden Anteil an der Vielfalt der marinen Organismen und der Fischerei, durch ihren mengenmäßigen Beitrag an Nahrung sowie die Bereitstellung günstiger Lebensräume für Tiere, von Bedeutung für den Schutz der Küsten vor Erosion während heftiger Gezeiten. Diese wirtschaftlich bedeutenden Nutzungen der Mangroven weisen darauf hin, dass sie im Leben und der Wirtschaft der Küstenregionen vieler Länder eine wichtige Rolle spielen. Mangrovenwälder sind ihrer vielfältigen wirtschaftlichen Nutzungen wegen sowie ihre Veränderungen durch Süßwasserzufuhr infolge verschiedener Tätigkeiten im Einzugsgebiet weltweit hochgradig gefährdet. Die Mangroven Pflanzen mit ihren einzigartigen Anpassungen spielen eine herausragende Rolle in der Erhaltung des Lebens der Mangrovenwälder. Ihre Reproduktionsbiologie ist von zentraler Bedeutung für das Verständnis der strukturellen und funktionellen Komponenten von Mangrovenwäldern.

Der Erfolg der sexuellen Vermehrung und die darauffolgende Ausbreitung der Populationen von Mangroven-Pflanzen führt zur zeitlichen Verknüpfung von Blüte, Bestäuber und Gezeitenströmungen. Lebendgebährende und versteckt-lebendgebährende Pflanzen sind echte Mangrovenarten während nicht lebendgebährende Arten den Magrovenpflanzen beigesellt sind. Die Verbreitungseinheit sind die Keimlinge bei lebendgebährenden und nicht-lebendgebährenden Arten, da es ein Same in nicht lebendgebährenden Pflanzen ist. In diese Studie wurden lebengebährende und Krypto-lebendgebährende Arten einbezogen. Diese Arten sind selbst-kompatibel, Selbstbestäuber und auch Fremdbestäuber; ein solches Aufzucht System ist eine Voraussetzung für den Erfolg der sexuellen Fortpflanzung sowie den anschließenden Aufbau und die Ausbreitung der Population. Im Untersuchungsgebiet sind sie entomophil. Die lebendgebährenden Arten umfassen *Ceriops tagal, C. decandra, Rhizophora apiculata, R. mucronata, Bruguiera gymnorrhiza* und *B. cylindrica*. Zu den nicht-lebendgebährenden Arten gehören *Avicennia alba, A. marina, A. officinalis, Aegiceras corniculatum* und *Aegialitis rotundifolia*. Sexuelle Fortpflanzung und Regeneration gehören zu den jährlichen Ereignissen in diesen Pflanzen und sind abhängig von vorort lebenden Insekten, Gezeitenströmungen sowie Nährstoffgehalt im Bereich der Ästuare.

In der letzten Zeit, haben unregelmäßige und unzureichende Regenfälle zusammen mit Einleitung von industriellen Schadstoffen Wachstum, Entwicklung und Regeneration der Mangrovenflora negativ beeinflusst. In der Tat ist ein allmählicher Rückgang der Mangrovenflächen festzustellen. Hinzu kommt eine kontinuierliche Nutzung der Mangroven für Brennholz, Bau von Unterkünften für Rinder sowie Flächeninanspruchnahme für Industriebetriebe und Entwicklung der Aquakultur in Flussmündungsgebieten. Als Folge hat die vorhandene Mangrovenfläche Schwierigkeiten zu überleben und ist auch nicht in der Lage, lokale Bedürfnisse zu unterstützen und die Möglichkeit für die Bereitstellung einer Existenzgrundlage durch Fischbestände sicher zu stellen. Ferner haben die reduzierten Mangrovenbestände katastrophale Auswirkungen auf die entlang der Küstelinie vom Fischfang lebenden Gemeinden, während Wirbelsturmbrandungen und Tsunami Ereignissen.

REZUMAT: Ecologia reproductivă a florei de mangrove: conservare şi management.

Mangrovele sunt ecosisteme dinamice şi unice de maree, comune în zonele tropicale şi subtropicale de coastă. Acestea sunt printre cele mai productive ecosisteme din lume și sunt importante în protejarea coastelor împotriva eroziunii în caz de maree excepţionale, în promovarea diversității de organisme marine și a pescuitului, a punerii la dispoziţie a unei cantităţi de produse alimentare și a furnizării de habitate favorabile pentru animale. Aceste utilizări economice ale mangrovelor indică faptul că acestea joacă un rol important în viața și în economiile din regiunile de coastă din diferite țări. Pădurile de mangrove sunt în mare pericol la nivel mondial, datorită multiplelor utilizări economice și a modificărilor cauzate de intrări de apă dulce în urma unor activităţi din amonte, în bazinul râurilor. Plantele de mangrove, cu adaptări unice, joacă un rol esențial în susținerea vieții în pădurile de mangrove. Biologia lor de reproducere este esențială pentru înțelegerea componentelor structurale și funcționale ale pădurilor de mangrove.

Succesul de reproducere sexuală și extinderea ulterioară a populației de mangrove este legat de perioadele de înflorire, de polenizatori și de curenții mareelor. Plantele vivipare și cripto-vivipare sunt mangrove adevărate, iar cele non-vivipare sunt specii asociate de mangrove. Dispersarea propagulelor se face prin răsaduri la plantele vivipare și non-vivipare și totodată acesta reprezintă sământa pentru plantele non-vivipare. În prezenta lucrare au fost incluse specii vivipare și cripto-vivipare. Aceste specii sunt auto-compatibile, auto-polenizatoare și, de asemenea, polenizate de alte specii. Un astfel de sistem de reproducere este o cerință pentru succesul reproducerii sexuate şi ulterior pentru dezvoltarea și extinderea populației. Ele sunt entomofile în regiunea de studiu. Plantele vivipare includ speciile *Ceriops tagal, C. decandra, Rhizophora apiculata, R. mucronata, Bruguiera gymnorrhiza* și *B. cylindrica*. Plantele non-vivipare includ *Avicennia alba, A. marina, A. officinalis, Aegiceras corniculatum* și *Aegialitis rotundifolia*. Reproducerea sexuată și procesele de regenerare sunt anuale la aceste plante și sunt dependente de insecte locale, curenții mareelor și conținutul de nutrienți în mediul de estuar.

În ultima vreme, ploile neregulate și insuficiente, împreună cu poluanții industriali eliberaţi în râuri, au influenţe negative asupra creşterii, dezvoltării și regenerării mangrovelor. Într-adevăr, există o scădere treptată a acoperirii cu mangrove. La aceasta se adaugă exploatarea continuă de plante de mangrove pentru lemn de foc, crearea de adăposturi pentru vite și schimbări în vederea amplasării de unități industriale și dezvoltarea acvaculturii în regiunile estuarine. Ca urmare, suprafaţa acoperită de mangrove prezintă dificultăţi de supraviețuire și, de asemenea, nu este capabilă să satisfacă nevoile locale și să ofere oportunități de trai prin intermediul resurselor de pescuit. Mai mult, suprafaţa redusă de mangrove are efecte catastrofale asupra comunităților de pescari care locuiesc de-a lungul liniei țărmului, în perioada ciclonică și a evenimentelor de tsunami.

INTRODUCTION

Mangrove plants are the key constituents and play a crucial role in sustaining life in mangrove forests. They display special adaptations in root system, shoot system, leaf characteristics and reproductive biology to live in the harsh environment (Tomlinson, 1986). Further, they exhibit peculiarities in seedling development and dispersal by way of vivipary. Vivipary is a functional characteristic defined as the precocious and continuous growth of the offspring when still attached to the maternal parent (Goebel, 1905); it is the norm in true mangrove plants (Tomlinson, 1986). There are two types of vivipary, true and crypto-vivipary.

True vivipary refers to a situation where embryo penetrates through the fruit pericarp and grows to a considerable size before dispersal, while crypto-vivipary is a condition in which the embryo grows continuously, but does not emerge from the fruit before dispersal. These peculiar seedling characteristics present in mangrove plants could be adaptive features to overcome the harsh tidal environment for seedling establishment (Elmqvist and Cox, 1996). Therefore, reproductive biology of mangrove plants is central to understanding the structural and functional components of mangrove forests. Further, this knowledge is essential for the restoration of degraded mangrove areas.

The focus on the reproductive biology of mangrove plants has almost exclusively been on the fruit, seed or seedling dispersal stage. Surprisingly, less is known about floral biology, pollination, breeding systems and success rate of propagule production, although knowledge of the effectiveness of floral mechanics and genetic isolating mechanisms is an important prerequisite to the study of successful dispersal and establishment (Juncosa and Tomlinson, 1987; Clarke and Meyerscough, 1991; Ge et al., 2003; Chiou-Rong et al., 2005; Coupland et al., 2006). In India, a few studies provide some preliminary accounts of floral biology and pollination in some mangrove plants (Solomon Raju, 1989; Solomon Raju et al., 1994; Subba Reddi and Solomon Raju, 1997; Pandit and Choudhury, 2001; Solomon Raju et al., 2006; Solomon Raju and Jonathan, 2008). Therefore, a more complete understanding of the reproductive biology of mangroves is useful, mainly due to the growing pressures on mangroves from coastal environment and for effective mangrove rehabilitation programmes.

The present study is an attempt to provide information on the reproductive ecology of the genera *Ceriops, Rhizophora, Bruguiera* (viviparous), *Avicennia, Aegiceras, Aegialitis* and *Scyphiphora* (crypto-viviparous), *Excoecaria, Lumnitzera, Sonneratia, Xylocarpus, Brownlowia, Sarcolobus* and *Suaeda* (non-viviparous) occurring in Godavari and Krishna mangrove forests in the state of Andhra Pradesh, India. Floral biology, sexual system, breeding system, floral rewards, pollinators and their foraging behaviour have been investigated in these plant species. Further, fruit and seedling ecology, dispersal strategies and establishment have also been studied in these species. This knowledge is of high value for understanding the reproductive biology of the studied plant species and for conservation and management of these species. This knowledge is also useful for the restoration of degraded mangrove areas.

MATERIAL AND METHODS

Flowering phenology was examined by conducting field trips at selected short intervals for study on all chosen plant species. Inflorescence flowering phenology was recorded by tagging some inflorescences that have not anthesed yet and then following them daily until they ceased anthesis permanently. Daily anthesis schedules and anther dehiscence schedules were carefully observed. Intrafloral events were recorded chronologically by marking some flowers at bud stage on different conspecific plants. The aspects included flowering stage, flower organs wilting order, sepal/petal development, stamens and stigma stages and nectar production. Pollen output per anther/flower was determined as per the method given by Dafni et al. (2005). Ovule counting was made by crushing the pistil or splitting it gently and longitudinally with a blade on a glass slide spotted with lactophenol cotton blue and counting the ovules under a dissecting microscope. With the obtained pollen output and ovule number per flower, pollen-ovule ratio was determined. Stigma receptivity was examined visually and by H_2O_2 tests (Dafni et al., 2005). The amount of nectar secreted per flower was measured and expressed as µl of nectar/flower. The nectar sugar concentration was measured by using a Hand Sugar Refractometer (Erma, Japan) (Dafni et al., 2005). Nectar analysis for sugar types was done as per the paper chromatography method of Harborne

(1973). Nectar analysis for amino acid types was done as per the paper chromatography method of Baker and Baker (1973). Breeding systems were investigated by following the protocols given by Dafni et al. (2005). Different sets of inflorescences prior to their flowering were used for determining natural fruit set rates.

Foraging schedule and forage type collected by flower visitors were observed following the methods of Solomon Raju (1989). Observations on foraging behaviour versus pollination by flower visitors were made visually. To judge the foraging activity of flower visitors, the total foraging visits of each species for the day on certain plant species were expressed in percentage. Based on this data, the percentage of foraging visits made by each species for the day was calculated. Field observations were also made regarding seed dispersal modes and subsequent establishment in mangrove forest.

RESULTS

Ceriops tagal (Rhizophoraceae). It is an evergreen shrub/tree that flowers during November-February. The flowers are born in condensed short-stalked cymes formed from dichotomizing panicles, which arise from the axils of leaves on the terminal nodes of new shoots. Flowers are short-stalked, small, white, cup-shaped, strongly fragrant, bisexual and zygomorphic. Sepals are five, small, yellowish green, valvate enclosing the inner parts until anthesis and not reflexed after anthesis. Petals are five, free, white, pubescent, two lobed, and alternating with the sepals. The lower margins of adjacent petals are held together by patches of tightly intertwining, helically coiled hairs. Each petal has three distinct clavate appendages on its distal margins. Stamens are ten, five of them antisepalous, five others antipetalous and all ten inserted on the rim of the calyx cup. Each petal encloses the antipetalous stamen and an adjacent antisepalous stamen; the two stamens remain in the petal under tension enclosed above by the clavate appendages even after anthesis. Style is slender and terminated into minute separate stigmatic lobes. The stigma stands at the height of the stamens. Disc within the stamen ring is well developed and anther lobes enclose the base of the thick filaments. Ovary is semi-inferior, three-carpelled and three-locular with a total of six ovules.

The mature buds open between 16^{30}-18^{00} h. The calyx lobes separate at anthesis and diverge to expose the petals. The petals with the stamens inside, two per petal do not unfold naturally throughout the flower life, but remain in a tension with the spring-loaded stamens hooded above by clavate appendages. Anther dehiscence occurs in the bud. Pollen grains are triangular, light yellow, exine smooth and 15 µ in diameter. A flower produces 14,681 ± 25.62 pollen grains. The pollen-ovule ratio is 2,446:1. The stigma attains receptivity on the second day and remains receptive up to six days. But, peak receptivity occurs in third-fifth day. In this period, the white petals turn red gradually from the top to the base. A flower produces 5.65 ± 1.0 µl of nectar. The nectar sugar concentration is 35-50% and the common sugars include fructose, sucrose and dextrose with the first relatively more dominant. The nectar contains 12 amino acids which include tyrosine, glycine, methionine, proline, lysine, aspartic acid, glutamic acid, serine, cysteine, alanine, threonine and arginine. Of these, glycine, serine, cysteine, alanine and threonine are relatively dominant. The amino acids such as phenylalanine, valine, leucine, iso-leucine, tryptophan and histidine were not found in the nectar. The unpollinated flowers fall off on the seventh day. In pollinated flowers, the petals, stamens, the style and stigma drop off in this order in three-four weeks, while the fruit is in a growing stage. The sepals are persistent, become warty and spiny gradually and remain on the plant even after the shedding of propagules.

The buds produced proceed to open without abortion. In open flowers, abortion rate is 42%. Of the twenty inflorescences bagged, eight flowers set fruits and, thus, the fruit set in bagged flowers is 3%. Of the sixty four open inflorescences tagged, only 115 flowers set fruit, constituting 16.3% natural fruit set. Fruit set per inflorescence varied from one to five but one and two-fruited inflorescences were more common. The pollinated flowers take four weeks to produce mature fruits with only one seed. Fruits are conical by the extrusion of the upper part of the ovary, with brown and roughened surface. The seed has no dormancy and produces hypocotyls while on the mother tree in a span of about two months. The cotyledonary yellow cylindrical collar appears from the fruit about ten days prior to detachment of the hypocotyls. The hypocotyl is 26 cm long, distinctly ridged and hangs downwards. It is initially green, after the development of the collar, it shows a gradual colour change to brownish purple from hypocotyl end to plumule. The mature hypocotyls separate from the fruit, leaving the latter attached to the mother plant. The detached hypocotyls were found to settle in the vicinity of the mother plant.

The flowers do not expose the stamens naturally but the latter attains tensed condition in the delicate petals for release by a delicate external touch. The foragers included honeybees, *Apis cerana indica, A. florea,* the fly, *Chrysomya megacephala* and the butterfly *Tirumala limniace*. They showed foraging activity throughout the day but with varying percentage of foraging visits and also species-wise, the percentage of foraging varied (Fig. 1).

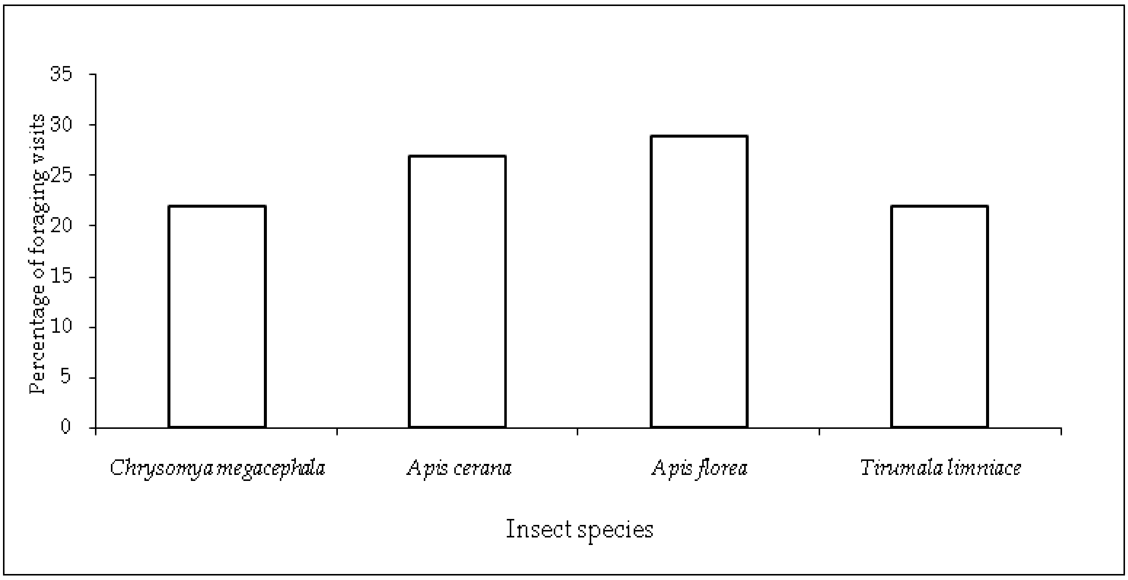

Figure 1: Percentage of foraging visits of insect species on *Ceriops tagal.*

Of these, honeybees foraged for pollen and nectar occasionally while the fly and butterfly foraged for the nectar consistently until the floral source exhausted. All the species approached the flower from above and probed for nectar, causing a sudden release of stamens from the petals. In effect, the pollen from the already dehiscent anthers was ejected forcibly and deposited on the underside of the foraging bee or fly. Body washings for pollen revealed the presence of pollen grains which varied from 231 to 413 per bee and from 79 to 147 per fly, suggesting that both bees and the flies have an important role in petal explosion and pollination. In case of bees, they also carried pollen loads in pollen baskets present on their legs. As each petal is independently enclosing two stamens, a single foraging visit of the bee or fly did not result in the explosion of all five petals. Both the bees and the fly tended to visit more than one flower on the same inflorescence or different inflorescences on the same plant before flying away to visit the neighbouring trees and may return back again to visit the same flowers later. As they tended to move back and forth between trees, their foraging activity may result in substantial self- and cross-pollination. The honey bees were found to concentrate principally on *Bruguiera gymnorrhiza* and *Aegiceras corniculatum*, while the fly species exclusively on *Ceriops tagal*. The explosion of petals in open flowers was also triggered by the action of wind. The plant grows in the seaward zone and hence high winds are the characteristic of the site. Of the twenty four flowers observed, four flowers had shown petal explosion triggered by wind action indicating that 16.6% of flowers may achieve pollination by wind action. In such flowers also, the explosion of all five petals did not take place at one time. It had not been possible to study whether wind could trigger petal explosion in the flowers located on the branches facing landward direction and mixed with the canopy where wind becomes relatively ineffective. Wind triggered petal explosion may result in autogamy.

Ceriops decandra (Rhizophoraceae). It is an evergreen, semi-understory, and small to moderate tall tree with perfect flowers. It produces numerous branches from the main stem. A typical tree of 1.5 meters contains as many as 20-21 primary branches and each primary branch bears 11-15 secondary branches. It occurs mainly in the inside of the mangrove vegetation and certain individuals occur along the creeks/estuarine banks. The tree shows budding, flowering and fruiting continuously throughout the year but there is a burst of concentrated flowering during November. Some young trees show alternate flowering and fruiting phases. The production rate of flowers and fruits are more robust on trees growing in the interiors of the forest when compared to those on trees growing along the creeks/estuarine banks. The flowers are borne in condensed cymes formed from dichotomizing panicles, which arise from the axils of leaves. The mechanical and biological features of this type of inflorescence provide continuous protection for the youngest units by a successive series of bracts, bracteoles and sepals. An inflorescence produces 5-31 flowers depending on the number of divisions of inflorescence axes. The cymes with more number of flowers are more common on the trees growing in the inner mangrove vegetation. The inflorescence takes six-ten days to complete its flowering life. Flowers are small, white, cup-shaped, odorless, bisexual and zygomorphic. Sepals are five, free, small, light green, six mm long, valvate enclosing the inner parts until anthesis and not reflexed after anthesis. Petals are five, free, two-lobed, alternating with the sepals, five mm long, light green in mature bud stage and white at anthesis. They are inter-locked marginally by basal short hairs and this circumstance produces a short corolla tube crowned by the series of clavate filamentous appendages. Stamens are ten, five of them antesepalous, five others antepetalous and all ten inserted on the rim of the calyx cup. Each stamen is two mm long, free and extends beyond the height of the stigma; the filaments are green, while anthers are light brown, dorsifixed and longer than filaments. Disc within the stamen ring is well developed and anther lobes enclosing the base of the stamens. The ovary is semi-inferior, three-carpelled and three-locular with a total of six ovules. Style is slender, green, one mm long and terminated into minute separate stigmatic lobes.

The mature buds open during 04^{30}-11^{00} h. The calyx lobes separate at anthesis and diverge to expose the petals, which adopt various configurations. Anther dehiscence occurs at anthesis. Pollen grains are ovate, triangular, light yellow and 16.6 µm in diameter. A flower produces 12,810 $\pm$ 30.87 pollen grains. The pollen-ovule ratio is 2,135:1. The stigma attains receptivity about six hours prior to anthesis and remains receptive up to six days; but very active receptivity occurs in one-day and two-day old flowers. The stigma receptivity is notable even after six days but that receptivity appears to be non-functional in terms of pollen germination. Nectar is produced in trace amounts. The petals and stamens fall off on the seventh day of flower life. The sepals are persistent, become warty and spiny gradually and remain on the tree even after the shedding of propagules. The style and stigma dry up and drop off after ten days in fertilized flowers. The flowers that were not pollinated fall off on the fourth day.

Hand pollination tests for breeding system indicated that *C. decandra* fruits through xenogamy only; the fruit set rate is 92%. Bud and flower abortion rate is 42% and 31% respectively. In open-pollinations, the fertilized flowers did not show a premature fruit drop. Fruit set per inflorescence varies from one-fur but not fruited inflorescences are more common. The pollinated flowers produce mature fruits in 50-55 days. Fruits are light green, 1.5 cm long, ovoid, conical and blunt apically. They are distinct with five-lobed persistent calyx and produce a single seed only. The embryo has no dormancy and penetrates through the seed coat and the fruit pericarp and grows to a considerable size into a spindle-shaped hypocotyl structure before dispersal while still attached to the maternal parent. This type of hypocotyl growth constitutes true vivipary. The hypocotyl grows upright and takes 85-90 days before detachment from the fruit. It is slender, clearly ribbed, angular, sulcate, 15 cm long and broadened at the lower end. Usually, they are entirely green and occasionally purple on one side; rarely yellow hypocotyls are also produced. The green hypocotyls seem to have the potential to photosynthesize actively with water and necessary nutrients drawn from the parent tree. The purple and yellow hypocotyls seem to lack chlorophyll partly or wholly and hence doubtful that they will have a successful establishment when detached from the parent tree. Very rarely, a single fruit produces two hypocotyls which may have arisen from two seeds resulting from two ovules out of actual six ovules per flower. In fully grown hypocotyls, fruit is separated from collar which emerges shortly before detachment. The fruit set rate per inflorescence shows a pattern in accordance with the number of flowers produced and area where the tree grows within the mangrove forest. An inflorescence produces one-six fruits. The trees growing near creeks/estuarine banks produce one-four fruits per inflorescence, one-fruited ones being 51%, two-fruited 34%, three-fruited 12% and four-fruited 3%. The trees growing in the inner mangrove areas produce one-six fruits per inflorescence, two-fruited ones are 45%, three-fruited 26%, one-fruited 22%, four-fruited 5% and five-fruited and six-fruited, 1% each.

The foragers included *Nomia* sp., *Ceratina simillima* (bees), *Odynerus* sp. and *Polistes* sp. (wasps). The bees were quite common during September-October, while the wasps during November-December. The bees were found to collect nectar and pollen, while the wasps only nectar. These foragers visited the flowers during 10^{30}-16^{30} h, more frequently during 13^{00}-14^{00} h coinciding well with the availability of the number of flowers, because anthesis period is relatively lengthy and the new flowers with nectar and pollen accumulate by that time. *Nomia* and *Odynerus* species made more percentage of foraging visits (Fig. 2). Bees approached the flowers in an upright position, landed in the cup-shaped flowers and probed for nectar and pollen in the same or different foraging visits. In doing so, their ventral side contacted the stamens first and then stigma effecting pollination. During pollen collection, these bees rotated around the flower to collect pollen from the anthers, which are situated in one whorl against the sepals and petals. They took three-five seconds to collect the forage from each newly opened flowers and two-three seconds from differently aged flowers. Their body washings for pollen revealed that they carry 1262.7 ± 428.7 (Range 35-1570) pollen grains and hence have an important role in pollination. In addition to this pollen, they also carried pollen loads in pollen baskets present on their legs. The bees were found to move between trees of *C. decandra* to collect both nectar and pollen and this inter-tree movement was considered to effect cross-pollination. As the number of new flowers per day is small in number, the bees were forced to collect forage from different trees of *C. decandra* and this flowering strategy is expected to promote cross-pollination. Wasps are about the size of a fly and construct their nest cells in sand heaps or in cavities of trees, lining them with agglutinated grains of sand or mud. The female wasps are known to feed on floral nectar after mating for the maturation of the eggs. The wasps observed on *C. decandra* collect nectar and such wasps were considered to be females. The study shows that *C. decandra* is a potential nectar source for female wasps. Wasps approached *C. decandra* flowers in an upright position, landed on the cymes, gradually moved to individual flowers and probed for nectar. They were found to move between individual trees in quest of more nectar. While probing the flower, they contacted the stamens and stigma with their underside and this resulted in pollination. The body washings for pollen indicated that they are pollen carriers; the pollen carry over ranges of eight to 49. The number of bees and wasps visiting the flowers vary in different months, but both species occur continuously. Considering the changing number of bees and wasps in different months, both categories seem to be equally important as pollinators. The stigmatic pollen loads were analyzed to evaluate the rate of pollen deposition per stigma. The analysis indicated that each stigma receives 40.60 + 37.21 (Range 5-170) pollen grains in open-pollinations but how much of it is xenogamic pollen is not known. The pollen deposition rate from the flowers was also analyzed to evaluate the role of foragers (after four-five flower visits) to empty the anthers and subsequent transportation to the receptive stigmas. The analysis showed that 10,243.7 + 1735.6 (Range 7660-12,400) pollen grains were depleted against the pollen output by 12,810 per flower suggesting that both bees and wasps are capable of transferring pollen and effecting pollination very effectively.

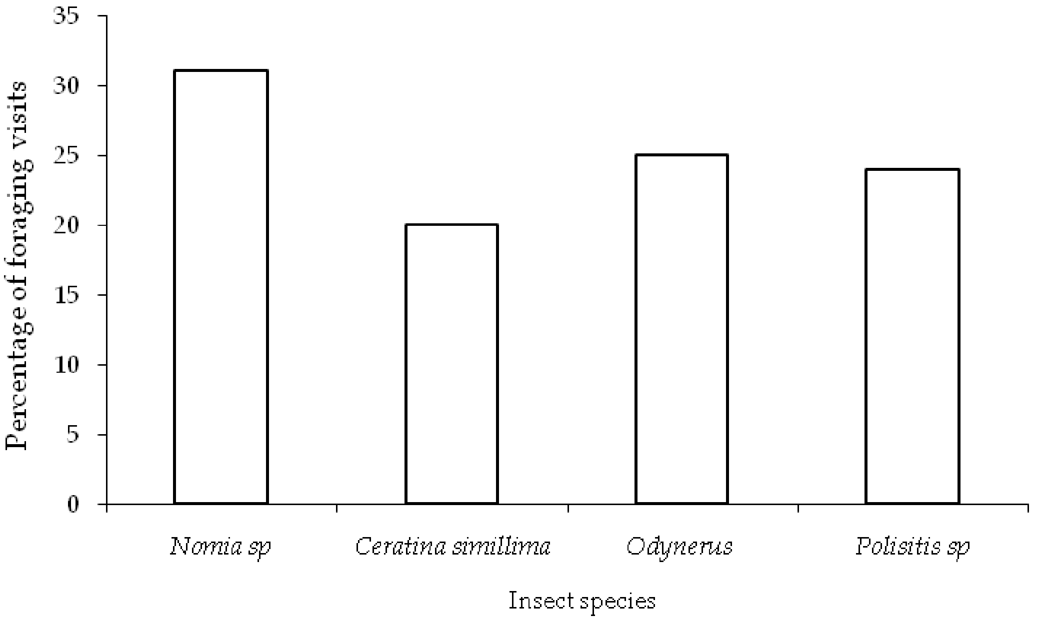

Figure 2: Percentage of foraging visits of insects on *Ceriops decandra*.

Field studies indicated that *C. decandra* is used as firewood and for the constructions. Further, this species is important for its reddish brown coloured bark. The bark is known for its high tannin content ranging from 68 to 75% and dyeing with this tannin gives brown colour. The fishermen extract a reddish liquid from the bark and use it to protect cotton fishing nets from decay for a longer period. Some fishermen are involved in the trade of this bark and wood to make up their livelihood. About 80% of fishermen in 30 shore-based villages adjacent to the Godavari Delta mangrove forests depend on fishing activity for their livelihoods. They use mostly cotton nets for catching fishery sources in backwaters, while nylon nets for fishing activity in open sea water. The advantages of cotton nets include inexpensiveness and readily available because they are made locally. Nylon nets are not readily available and they are also expensive. The treatment of cotton nets is necessary to prevent damage to cotton threads due to soaking in saline water and to extend the length of its durability.

Rhizophora apiculata (Rhizophoraceae). It is a medium to tall evergreen tree with profusely spreading branches, growing to a height of more than 10-12 m. The leaves are large and dark glossy green. It flowers throughout the year with profuse flowering during August-September. Flowers are borne in pairs on a stubby axis, borne below the leafy crown, that is, in the axil of a leaf scar. They are sessile and erect in position. Flowers are light yellow, 8-12 mm long, 10-14 mm wide, cup-shaped, odourless, bisexual and zygomorphic; they are situated below the leaf clusters. Calyx is characteristically hard, yellow to brown, six-eight mm long and three-four mm wide, basally cup-like, with four sepals pointed towards apex and persistent. Petals are four, alternating with sepals, five-seven mm long, creamy-white, odourless, lanceolate, glabrous and delicate. Stamens are 12, sessile, dull white to dull brown, free, anthers are five-six mm long, bilobed, introrse and sagittate. The ovary is inferior, globose with two carpels each with two glabrous ovules on axile placentation; style is thick and short, creamy white, with two creamy white to light pink stigmatic lobes.

The mature buds open at 10^{00}-11^{00} h; the sepals diverge least but expose the inconspicuous petals and sex organs. The petals remain flat and do not recurve. The anthers are multicellar, dehisce introrsely via the adaxial flap which falls against the base of the style in mature bud stage. The glabrous petals do not accumulate pollen from the dehisced anthers. Stigmatic lobes are appressed in mature bud and diverged gradually after anthesis; the diverged state of stigmatic lobes indicates the commencement of receptivity and ceases around noon of the second day. The stigma has no special modifications to capture the wind borne pollen but it is thickly coated with pollen even in mature bud. The style and stigma fall off after fruit initiation. The stamens and petals drop off on the second day. The calyx remains attached to the growing fruit, expands and reflexes backward. The pollen output per anther is 46,527.1 ± 2,411.9 (Range 42,681-49,854) and per flower is 5.58.326. Pollen grains are tricolporate, dull-white, powdery, ornamentation finely reticulate, the reticulum becoming progressively less distinct from pole to equator and 16.6 µm diameter. The pollen-ovule ratio is 1.39.581:1. The pollen protein content per anther is 8.6 µg and per flower is 104 µg. Nectar is secreted in trace amount around the ovary. The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is 72.5% in spontaneous autogamy, 86% in hand-pollinated autogamy, 92% in geitonogamy, 93.3% in xenogamy and 55% in open pollination.

The flowers release pollen into the air; the sepals and petals in open flowers sprinkled with pollen due to wind action. A delicate manual disturbance to the flowers resulted that caused the release of a cloud of pollen out of the flower. Bees were the exclusive foragers and they were *Nomia* sp., *Trigona iridipennis* and *Halictus ligatus.* They foraged during day time from 08^{00}-17^{00} h for pollen and nectar from partially and completely opened flowers. Their foraging activity was consistent during profuse flowering period while it is sporadic at other times of the year. Of the total number of foraging visits of bees, *H. ligatus* made 44% followed by *Nomia* 41% and *Trigona* 15% (Fig. 3). These bees first landed on the sepals, then investigated the flower for pollen and nectar; while doing so, their head and ventral side of the body touched the stamens and stigma and in the process they got coated with pollen. Further, they loaded pollen into pollen baskets. As all three bee species are pollen collecting bees, they made frequent visits to flowers situated on different plants which are closely and distantly spaced in order to collect more pollen. The fresh flowers available per day on a given tree are small in number and the bees made visits to different trees to collect more forage from as many flowers as possible. Further, the body washings of the bee species revealed the presence of pollen; the average number of pollen grains per bee for each species varied from 561.6 to 1006.9. It was found that thrips breed in buds and emerge when a flower opening occurs. They collected both pollen and nectar. During August-September, a single mature bud was usually found to contain many thrips moving out of the floral base when disturbed manually. Thrips were found to effect pollination as they moved in the entire flower touching the stigma.

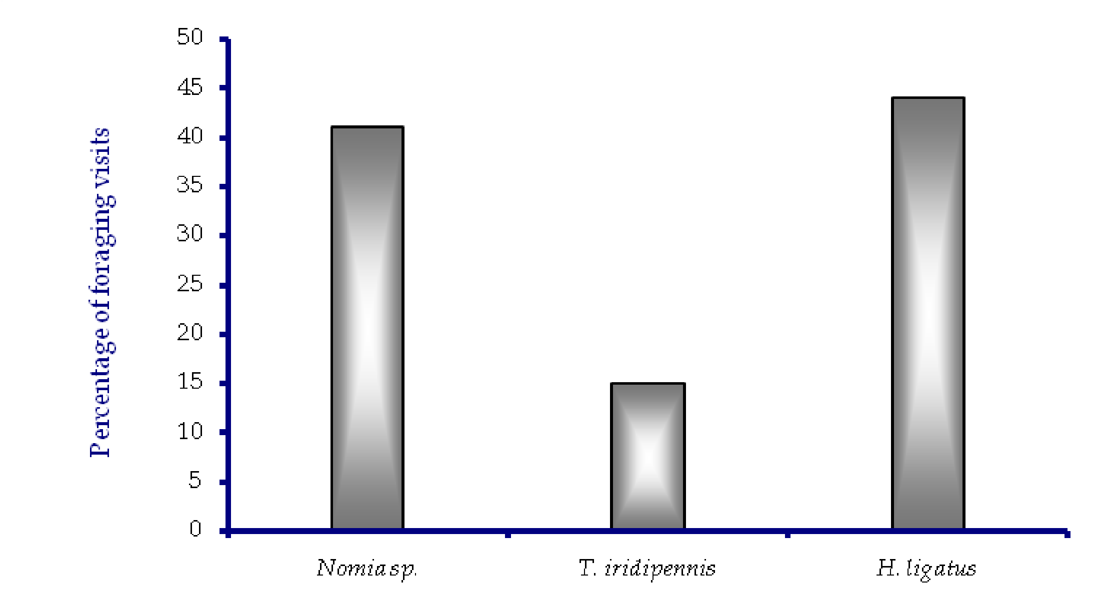

Figure 3: Percentage of foraging visits of bees on *Rhizophora apiculata*.

Pollinated and fertilized flowers initiate fruit development immediately and take 35 days to produce mature fruits. Of the four ovules in a flower, one ovule produces seed. Fruit is one-seeded with persistent light green calyx. A fruit producing two seeds and hence two hypocotyls is a rare occurrence. The seed produces 550 mm long, cylindrical elongate, light green, hypocotyl with a sharp end in a time span of 55-60 days. A light brown collar emerges between fruit and hypocotyl about a month prior to the detachment of the latter. The persistent calyx and fruit part remain in place on the parent tree while the hypocotyl detaches at the collar for dispersal. The hypocotyls fall and anchor vertically in the substratum at low tide when the forest floor gets exposed; they float in water and disperse by tidal currents at high tide until settled in the mud. The radicle side of hypocotyl penetrates the soil and produces root system while plumule side produces new leaves and subsequent aerial system.

Rhizophora mucronata (Rhizophoraceae). It is a medium to tall evergreen tree with profusely and horizontally spreading branches, growing to a height of more than 15 m. It flowers during June-November. The inflorescence is a four-flowered pedunculate dichotomized cyme borne in leaf axils; the flowers are pedicellate, pendulous and hanging downwards. Flowers are creamy white, 15 mm long, 10 mm wide, cup-shaped, odourless, bisexual and zygomorphic; they are situated below the leaf clusters. Calyx is characteristically hard, creamy white, 12 mm long and seven mm wide, basally cup-like, with four sepals pointed towards apex and persistent. Petals are four, alternating with sepals, ten mm long, white, hairy, odourless, lanceolate, and delicate. Stamens are eight, sessile, four opposite to petals, another four opposite to sepals, dull white to dull brown, free, anthers are seven mm long, bilobed, introrse and sagittate. Ovary is semi-inferior, globose with two carpels each with two glabrous anatropous ovules on axile placentation; style is thick and short, creamy white, with two creamy white to light pink stigmatic lobes.

The mature buds open at 10^{00}-16^{00} h; the sepals diverge gradually exposing the petals and sex organs. The petals also diverge and slightly reflex backwards. The anthers are multilocellar, dehisce introrsely via the adaxial flap which falls against the base of the style in mature bud stage. The hairy petals accumulate pollen from the dehisced anthers. Stigmatic lobes are appressed in mature bud and diverged gradually after anthesis; the diverged state of stigmatic lobes indicates the commencement of receptivity and ceases around noon of the second day. The stigma has no special modifications to capture the wind borne pollen, but it is thickly coated with pollen even in mature bud. The style and stigma fall off after fruit initiation. The stamens and petals drop off on the second day. The calyx remains attached to the growing fruit, expands and reflex backwards.

The pollen output per anther is 34,986.6 ± 527.34 (Range 34,007-35,729) and per flower is 2,79,893. Pollen grains are tricolporate, dull-white, powdery, and 24.9 µm long and 12.2 µm in diameter; their surface sculpture is rather smooth, with numerous small and shallow depressions. The pollen-ovule ratio is 69,973.2:1. The pollen protein content per anther is 10.2 µg and per flower is 82 µg. Nectar volume per flower is 1.03 ± 0.26 µl (Range 0.8-1.5) and accumulated around the ovary. The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is 52.5% in spontaneous autogamy, 63.3% in hand-pollinated autogamy, 71.4% in geitonogamy, 85% in xenogamy and 53.3% in open pollination.

The flowers release pollen into the air; the sepals and petals in open flowers were found to be sprinkled with pollen due to wind action. A delicate manual disturbance to the flowers resulted caused the release of a cloud of pollen out of the flower. The flowers were foraged consistently during day time from 08^{00}-17^{00} h exclusively by bees. The bees included *Nomia* sp., *Trigona iridipennis*, *Halictus ligatus*, *Ceratina simillima* and *Xylocopa pubescens*. An unidentified moth was also found to collect nectar occasionally. Bees collected both pollen and nectar from partially and completely opened flowers. Each bee species contributed 11-24% of total foraging visits (Fig. 4). These bees first landed on the sepals, then entered the flower to collect pollen and nectar; while doing so, their head and ventral side of the body touched the stamens and stigma and in the process they got coated with pollen. Further, they loaded pollen into pollen baskets. All the bee species except *Xylocopa*, are pollen collecting bees, they made frequent visits to flowers situated on different plants which are closely and distantly spaced in order to collect more pollen. *Xylocopa* bees are fast fliers and made inter-tree flight in quest of more nectar from as many flowers as possible. The fresh flowers available per day on a given tree are small in number and for this reason, the bees were compelled to make visits to different trees for more forage. Further, the body washings of the bee species revealed the presence of pollen; the average number of pollen grains per bee for each species varied from 189.3 to 951.6. Thrips breed in buds and emerge when flower opening occurs. They collected both pollen and nectar. A mature bud contained numerous thrips moving out of the floral base when disturbed manually. Thrips were found to carry pollen on their bodies and moving in the entire flower including the stigma during which they transferred pollen onto the stigma. The flowers with huge pollen production together with pollen characteristics described above were considered to be anemophilous. Further, the pollen being powdery was found to be dispersed easily due to wind action. A simple manual disturbance to flowers made the latter to release pollen into the air.

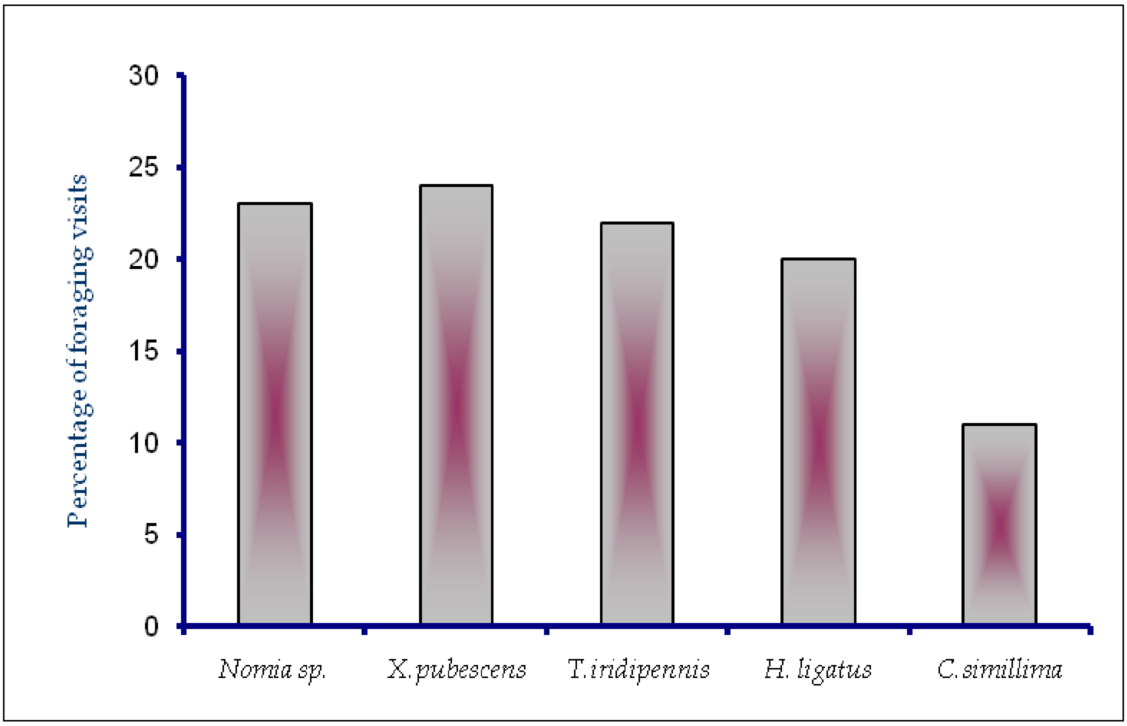

Figure 4: Percentage of foraging visits of bee on *Rhizophora mucronata*.

Pollinated and fertilized flowers initiate fruit development immediately and take 40 days to produce mature fruits. In fertilized flowers, only one ovule out of four ovules produced seeds. Fruit is one-seeded with woody, persistent light green and reflexed calyx. Seed produces 680 mm long, cylindrical elongate, light green, warty hanging hypocotyl in a time span of 60-65 days. A light yellow tubular collar (20 mm long) emerges between fruit and hypocotyl about a month prior to detachment of the latter. The hypocotyl detaches at the collar for dispersal. Later, the entire fruit part also falls off. The hypocotyls settle in the substratum immediately at low tide when the forest floor gets exposed; they float in water and disperse by tidal currents at high tide until settled in the soil. The radicle side of hypocotyl penetrates the soil and produces root system, while plumule side produces new leaves and subsequent aerial system. The fruit also falls off eventually from the maternal parent.

Bruguiera gymnorrhiza (Rhizophoraceae). It is a medium to tall evergreen tree with much diffused spreading branches, growing to a height of more than ten m. It flowers throughout the year with profuse flowering during April-June. Flowers are solitary, single flower in each peduncle, located in the leaf axils, usually positioned at the first (or rarely second) node below the apical shoot. Flowers are pedicellate, typically recurved, pointing away from the terminal vegetative bud, pendulous, 30-35 mm long, 19-20 mm wide, pinkish to reddish white, tubular, odourless, bisexual and zygomorphic. Calyx is characteristically hard, smooth or with grooves above lobe junctures, pinkish to reddish white, basally cup-like, rarely ribbed, with 11-14 lobes, acutely pointed, narrow and persistent. Petals are 14, bilobed, 15 mm long and four mm wide, creamy-white in mature bud, orange-brown on maturation, delicate with marginal interlocking hairs, the tips of lobes acute commonly with three filamentous appendages distally usually with a rigid straight four mm long bristle between them. The base of each petal has a cluster of smooth silky hairs. Stamens are 28 enclosed in petal pouches, two stamens in each petal, filament creamy white, ten mm long and two mm wide, anthers are creamy white initially and turn to golden brown at maturity; bilobed and basifixed. Ovary is inferior, cup-shaped with six light brown, smooth ovules; style is slender, white to light brown, filiform with three or four, 15 mm long, whitish-yellow stigmatic lobes containing small papilla and secreting mucilage; the stigma remains attached to fruit at maturity.

The mature buds open at 07^{00}-09^{00} h; the sepals diverge gradually presenting the closed, erect petals in the cocked position. The petals conceal the stamens in a tensed state due to the pressing of the latter against the interlocked margins of the petals. The petals bend back and remain in an erect position by the adherent ventral margins. Marginal hairs appear to be important in holding the petals in the folded position. The stamens dehisce in mature bud stage by longitudinal slits. The petal margins unzip instantaneously when triggered by external touch; then they fly apart releasing the stamens which catapult the loose pollen as a visible cloud toward the centre of the flower. If the external touch is caused by a forager, then much of the pollen would be projected onto the head and the body of the forager. Each petal explodes independently and hence multiple visits are required to trip all the petals of a flower. Individual flowers with combinations of closed and tripped petals were found. After the petal tripping, the petals lie back against the calyx lobes with empty stamens twisted and disorganized. Petals and stamens persist for up to seven days and eventually fall off as three-four units. Untripped petals retain their tension for up to eight days, and they eventually fall off without ever opening. Stigmatic lobes are receptive from second to fourth day and show signs of withering after the petals and stamens have fallen. The pollen output per anther is 9,005.4 ± 834.95 (Range 8,004-10,469) and per flower is 2,51,856 pollen grains. Pollen grains are tricolporate, pale yellow, elliptic in equatorial view, circular in polar view, exine smooth with numerous small and shallow depressions, 29.6 µm long and 16.6 µm diameter. The pollen-ovule ratio is 41,976:1. The pollen protein content per anther is 1.77 µg and per flower is 49.6 µg. Copious nectar accumulates in the deep calyx cup and is retained by the petal base and its associated hairs. In newly open flowers, five-ten µl of nectar is secreted and it is continuously produced even after the abscission of petals and stamens in some flowers while it is not produced continuously in some other flowers. The flowers with the former situation were designated as un-pollinated ones while those with the latter situation were designated as pollinated flowers. A flower produces 10.49 ± 5.2 (Range 5.2 - 24) µl of nectar with a sugar concentration of 23.48 ± 4.80% (Range 18-31); glucose and fructose were present. In unvisited flowers, it overflows onto the petals where it becomes viscous by evaporation and so inhibits the explosive mechanism. The total sugar content in the nectar of a flower is 2.58 ± 1.20 (Range 1.06-5.39) mg. The nectar protein content per flower is 78.68 ± 48.56 µg (Range 44.8-

192). The nectar contains eight amino acids which include alanine, aspartic acid, glutamic acid, arginine, histidine, lysine, glycine and serine. All these amino acids are abundant except lysine. The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is 40% in manipulated autogamy, 60% in geitonogamy, 90% in xenogamy and 87% in open pollination.

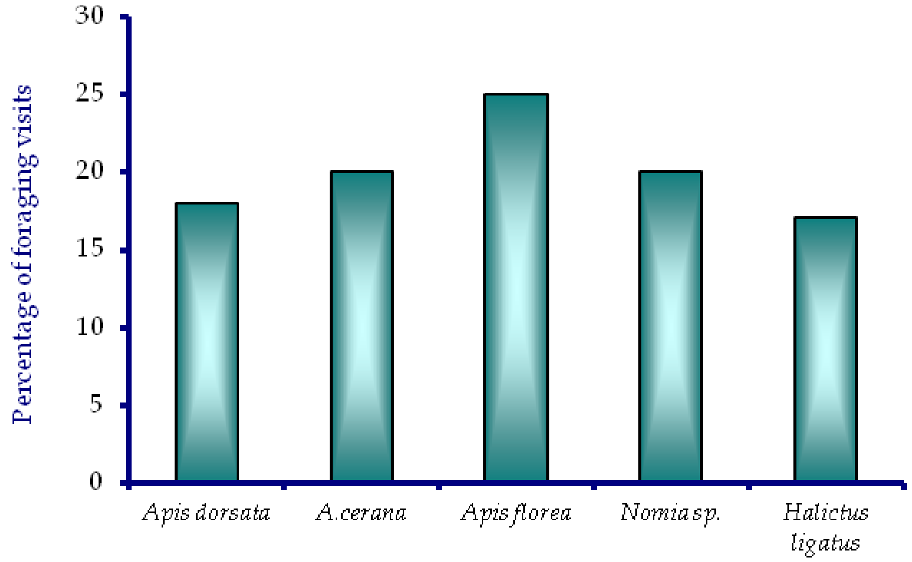

Figure 5: Percentage of foraging visits of bees on *Bruguierra gymnorrhiza*.

The flowers are characterized by explosive pollination mechanism. They were foraged consistently during day time from 07^{00}-17^{00} h exclusively by bees. The bees included *Apis dorsata, A. cerana, A. florea, Nomia* sp. and *Halictus ligatus*. Bees collected both pollen and nectar from partially and completely opened flowers. Of the total number of foraging visits of bees, *A. florea* contributed 25%, *A. cerana* and *Nomia* each 20%, *A. dorsata* 18% and *H. ligatus* 17% (Fig. 5). All the bees turned their heads upward to collect nectar located in the calyx cup; while doing so, they tripped the tensed petals to release a cloud of pollen explosively without being disturbed by the explosion. A single foraging visit did not result in tripping all the petals at a time. Petal explosion was found to be effected by touching sensitive basal hairs, especially those which project in the centre of the flower over the entrance to the calyx cup. The tip of the petal and its apical appendages were not sensitive but petal tripping was stimulated by fairly vigorous probing into the calyx cup. The bees took different positions while probing and collecting nectar in relation to stamens and style. Each foraging visit invariably contacted the style and stigma and resulted in the deposition of pollen on the foraging bee. The bees were also found to collect nectar from empty flowers which are devoid of petals and stamens. They were found to move frequently between plants seeking more nectar and/or pollen as few rewarding flowers are available daily per tree. Such a foraging behaviour could make them as effective pollen dispersers causing both self- and cross-pollination. Further, the body washings of all foraging bee species revealed the presence of pollen; the average number of pollen grains per bee for each species varied from 198 to 1,389.

Pollinated and fertilized flowers initiate fruit development immediately and take 30-35 days to produce mature fruits. Fruit is one-seeded fleshy berry with persistent reddish calyx. Seed produces 141 ± 9 mm long, cylindrical elongate, stocky, dark green, coriaceous hanging hypocotyl with blunt apex in a time span of 45-50 days. In a sample of 200 hypocotyls, 5% were found to be damaged especially at the terminal part by the Rose-ringed Parakeet, *Psittacula krameri*; the damaged part was fleshy and hence eaten by the bird. The persistent calyx remains attached even after mature hypocotyl falls from the mother tree. The hypocotyls settle in the substratum immediately at low tide when the forest floor is exposed; they float and disperse by tidal currents at high tide until settled in the soil. The radicle side of the hypocotyl penetrates the soil and produces root system while plumule side produces new leaves and aerial system.

Bruguiera cylindrica (Rhizophoraceae). It is a medium to tall evergreen tree with diffused spreading branches, growing to a height of more than 12 m. The flowering occurs during September-March. The inflorescence is a simple pedunculate dichasium cyme with pedicellate erect flowers; each cyme is three-flowered and borne in leaf axils. Flowers are greenish-white, 10-12 mm long, short-tubed, odourless, bisexual and zygomorphic. Calyx is tubular, funnel-like (five-seven mm long and two mm wide), smooth, light green, with seven-eight pointed lobes, characteristically hard and persistent. Petals are seven-eight, initially creamy white, later turning to light brown from the tip towards the base, alternating with sepals, shortly bilobed, each lobe three mm long and one mm wide, delicate with marginal interlocking minute hairs, the tips of lobes acute commonly with three filamentous appendages distally usually with a rigid straight bristle between them. The base of each petal has a cluster of smooth silky hairs. Each petal encloses a pair of stamens, filament creamy white, two mm long and one mm wide, anthers are creamy white initially and turn to light brown at maturity; bilobed and basifixed. Ovary is inferior, cup-shaped, with four light brown, smooth ovules; style is slender, white to light brown, filiform with two creamy white to yellow stigmatic lobes, two mm, situated below the height of stamens, stiff and remains attached to fruit at maturity.

The mature buds open at 07^{00}-09^{00} h; the sepals diverge gradually presenting the closed, erect petals in the cocked position. The petals conceal the stamens in tensed state due to the pressing of the latter against the interlocked margins of the petals. The petals are retained in an erect position by the adherent ventral margins. Marginal hairs appear to be important in holding the petals in the folded position. The stamens dehisce in mature bud stage by longitudinal slits. The petal margins unzip instantaneously when triggered by a delicate touch; then they fly apart releasing the stamens which catapult the loose pollen toward the centre of the flower. If the external touch is caused by a forager, then much of the pollen would be projected onto the head and body of the forager. Each petal explodes non-violently and independently. In an observed set of flowers, most flowers had all petals tripped. After the petal tripping, the petals lie back against the calyx lobes with the empty stamens twisted and disorganized. Petals and stamens persist for up to three days and eventually fall as three-four units. Untripped petals retain their tension for up to six days, and they eventually fall off without ever opening. Stigmatic lobes are receptive on the second and third day and show signs of withering after the petals and stamens have fallen. The pollen output per anther is 1,289.7 ± 299.29 (Range 1,021-1,828) and per flower is 20,635 pollen grains. Pollen grains are tricolporate, light yellow, powdery, ornamentation finely reticulate, the reticulum becoming progressively less distinct from pole to equator and 16.6 µm long. The pollen-ovule ratio is 5,158:1. The pollen protein content per anther is one µg and per flower is 16 µg. Nectar accumulates in the deep calyx cup and is retained by the petal base and its associated hairs. A flower produces 1.43 ± 0.31 µl (Range 0.9-2) of nectar with a sugar concentration of 15.2 ± 1.57% (Range 10-21); glucose, fructose, sucrose and maltose were present but the first sugar is dominant. The total sugar content in the nectar of a flower is 0.11 ± 0.03 (Range 0.04-0.17) mg. The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is 40% in unmanipulated autogamy, 56% in manipulated autogamy, 63.3% in geitonogamy, 80% in xenogamy and 64.4% in open pollination.

The flowers are characterized by explosive pollination mechanism. They were foraged consistently during 07^{00} to 17^{00} h by *Nomia* bee and the wasps, *Odynerus* sp. and *Polistes humilis*. The bee collected both pollen and nectar while the wasps collected only nectar. Of the number of foraging visits of bees, *Nomia* contributed 38% while the remaining percentage by the wasps (Fig. 6).

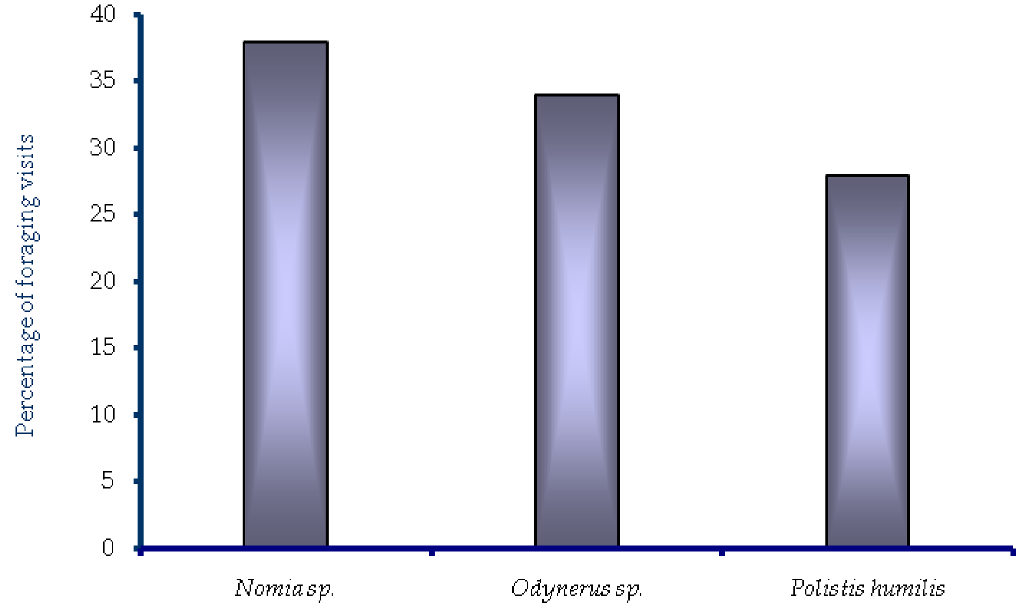

Figure 6: Percentage of foraging visits of insects on *Bruguiera cylindrica*.

These foragers approached the flowers in upright position and probed the flowers for nectar and/or pollen; they tripped the tensed petals without being disturbed by the explosion. One or two visits of the foragers resulted in the explosion of all flower petals. Petal explosion was found to be effected by touching sensitive basal hairs, especially those which project into the centre of flower over the entrance to the calyx cup. The tip of the petal and its apical appendages were not sensitive but petal tripping was stimulated by vigorous probing into the calyx cup. Each foraging visit invariably contacted the style and stigma and resulted in the deposition of pollen on the foraging bee. They were found to move frequently between plants seeking more nectar and/or pollen as few rewarding flowers are available daily per tree. Such a foraging behaviour could make them as effective pollen dispersers causing both self and cross pollination. Further, the body washings of all foragers revealed the presence of pollen; the average number of pollen grains per forager for each species varied from 262 to 448.2.

Pollinated and fertilized flowers initiate fruit development immediately and take 18-20 days to produce mature fruits. Of the four ovules, only one ovule produces seed in fertilized flowers. Rarely, two ovules produce seeds; in such fruits, twin hypocotyls are produced per fruit. Fruit is one-seeded, creamy white, 20 mm long; persistent creamy white calyx lobes stick out at right angles to the fruit. Seed produces 136 ± 16 mm long, cylindrical elongate, slightly curved, green to purple, pendulous hypocotyl with blunt apex in a time span of 30-35 days. The persistent calyx remains attached after mature hypocotyl falls from the parent tree. The hypocotyls settle in the mud at low tide during which the forest floor gets exposed; they float in water and disperse by tidal currents at high tide. The radicle side of the hypocotyl produces root system while plumule side produces new leaves and subsequent aerial system.

Avicennia alba (Avicenniaceae). Small evergreen tree with irregular branches, growing to a height of more than 10-12 m. Following monsoon showers in June, it initiates flowering and continues flowering until the end of August. Individual trees flower for 35 ± 4 (Range 32-48) days. Inflorescence is a terminal and axillary spicate raceme. An inflorescence produces, on average, 52.34 ± 26.96 flowers (Range 15-123) anthesing from the base to top over a period of 25 days (Range 24-28). The flowers are sessile, small (four mm long; three mm diameter), orange yellow, fragrant, actinomorphic and bisexual. Calyx is short, elliptic and has four ovate, green, pubescent, two mm long, one mm wide, sepals with hairs on the outer surface; it is persistent. Corolla has four thick, orange yellow ovate, four mm long and two mm wide petals forming a short tube at the base. Stamens are fur, epipetalous, one mm long, occur at the throat of the corolla. The anthers are dorsifixed, introrse and arranged alternate to petals. The ovary is very small (two mm long), flask-shaped, conspicuously hairy but lower part includes glandular hairs, bicarpellary syncarpous with four imperfect locules and each locule contains one pendulous ovule. It is terminated with a one mm long glabrous style tapered to the bifid hairy stigma. The light yellow style and stigma arise from the centre of the flower and stand erect throughout the flower life.

The mature buds open throughout the day but most buds opening during 09^{00}-12^{00} h. The petals slowly open and take three-four hours for complete opening to expose the stamens and stigma. The petals emit fragrance at anthesis. The stamens bend inward overarching the stigma at anthesis and dehisce ½ hour after anthesis. All the four anthers dehisce at the same time by longitudinal slits. The stigma is well seated in the centre of the flower. After anthesis, the stigma grows gradually and becomes bifid on the morning of the second day. The bifid condition of stigma is an indication of beginning of stigma receptivity and it remains receptive for two days. The stigmatic lobes recurve completely. A flower lasts for six days. The petals, stamens and stigma drop off while the calyx is persistent. The pollen production per anther is 1,967 ± 31.824.3 (Range 1,929-2,010) and per flower is 7,868. The pollen grains are light yellow, granular, tricolporate, reticulate, muri broad, flat, thick; lumina small irregularly shaped, colpi deeply intruding and 24.9 µm in size. The pollen-ovule ratio is 1,967:1. The flowers begin nectar secretion along with anther dehiscence. The nectar secretion occurs in minute amount which is accumulated at the ovary base and on the yellow part of petals; the nectar glitters against sunlight. A flower produces 0.5 ± 0.1 µl (Range 0.4-0.7) of nectar with a sugar concentration of 40%. The sugar types included glucose and fructose and sucrose with the first as dominant. The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is 17.5% in spontaneous autogamy, 40% in hand-pollinated autogamy, 62.5% in geitonogamy, 64.28% in xenogamy and 42% in open pollination.

The flowers were foraged consistently during day time from 07^{00}-17^{00} h by insects. The insects included *Apis dorsata, A. florea, Nomia* sp., *Chrysomya megacephala,* an unidentified fly, *Danaus chrysippus* and *Everes lacturnus*. The fly species visited the flowers in groups. The pollen was collected by bees only; they also collected nectar throughout the flower life depending on the availability. All other insects collected nectar only. Each forager species made 8.5-21% of foraging visits (Fig. 7). All the insects probed the flowers in upright position to collect the forage. Butterflies landed on the petals, stretched their proboscis to collect nectar aliquots on the petals and at the flower base. In this process, all the insects invariably touched the anthers and the stigma; the ventral side of all insects was found with pollen. Further, the body washings of the all insect species revealed the presence of pollen; the average number of pollen grains per insect for each species varied from 67.6 to 336.2. As the nectar is secreted in minute amount, the insects made multiple visits to most of the flowers on a tree and moved frequently between trees to collect nectar. Such a foraging behaviour was considered to effect self- and cross-pollination.

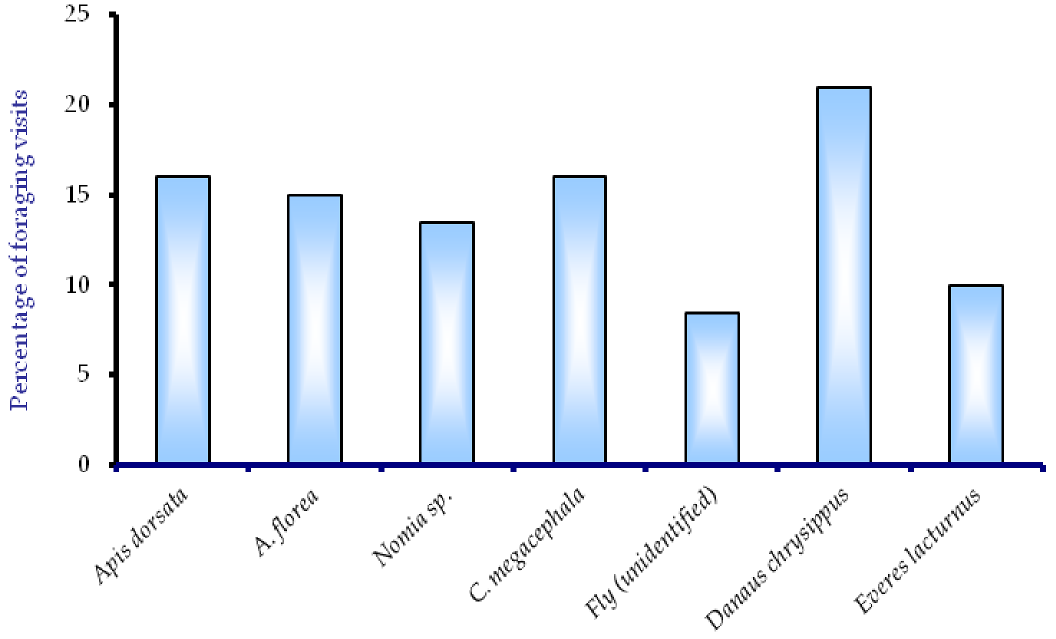

Figure 7: Percentage of foraging activity of insects on *Avicennia alba*.

Pollinated and fertilized flowers initiate fruit development immediately and take about five-six weeks to produce mature fruits. In fertilized flowers, only ovule produces seed. Fruit is a one-seeded leathery pale green capsule with persistent reddish brown calyx; 40 mm long, 15 mm wide, abruptly narrowed to a short beak and hairy throughout. Seed produces light green, hypocotyl which completely occupies the fruit cavity. An inflorescence produces three ± one fruits (Range 1-5); one-fruited ones constituted 54% followed by two-fruited ones (23%) and three-fruited (10%) and four-fruited (5%) and five-fruited (2%). Of these, 6% were damaged by the Rose-ringed Parakeet, *Psittacula krameri*; it was found to feed on the concealed hypocotyl in fruits and these fruits were subsequently empty. The fruit together with hypocotyl falls off the mother plant; it was found to settle in the substratum immediately at low tide period when the forest floor is exposed; it floats in water and disperses by tidal currents at high tide period until settled somewhere in the soil. The radicle side of hypocotyl penetrates the soil and produces root system while plumule side produces new leaves and subsequent aerial system. The fruit pericarp detaches and disintegrates when plumular leaves are produced.

Avicennia marina (Avicenniaceae). It is a small evergreen tree with irregular spreading branches, growing to a height of more than three-eight m. Following monsoon showers in June, it initiates flowering and continues flowering until the end of August. Individual trees flower in 32-35 days. Inflorescence is a compound axillary or terminal cyme. An inflorescence shoot produces, on average, 47 ± 13.97 flowers (Range 26-76) anthesing from the base to top over a period of 22 days (Range 15-22). The flowers are sessile, small (six mm long; five mm diameter), orange yellow, sweet scented, actinomorphic and bisexual. Calyx is short, elliptic and has four ovate light green, two mm long sepals with hairs on the outer surface; it is persistent. Corolla has four thick, orange yellow ovate four mm long and two mm wide petals forming a short tube at the base. The petals are glabrous inside and hairy outside. Stamens are four, epipetalous, two mm long, occur in the throat of the corolla. The anthers are basifixed, exserted, introrse and arranged alternate to petals. The ovary is very small (two mm long), conspicuously hairy, bicarpellary syncarpous with four imperfect locules and each locule contains one pendulous ovule. It is terminated with a one mm long glabrous style tapered to the bifid hairy stigma. The light yellow style and stigma arise from the center of the flower and stand erect throughout the flower life.

The mature buds open throughout the day but most buds open during 10^{00}-13^{00} h. The petals slowly open and take two-three hours for complete opening to expose the stamens and stigma. The petals emit a sweet fragrance at anthesis. The stamens bend inward overarching the stigma at anthesis and dehisce half an hour after anthesis. All the four anthers dehisce at the same time by longitudinal slits. The stigma is well seated in the center of the flower. After anthesis, the stigma grows gradually and becomes bifid on the morning of the second day. The bifid condition of stigma is an indication of beginning of stigma receptivity and it remains receptive for two days. The stigmatic lobes recurve completely. A flower lasts for five days. The petals, stamens and stigma drop off while the calyx is persistent. The pollen production per anther is 1643.2 ± 31.8 (Range 1600-1690) and per flower is 6572.8. The pollen grains are light yellow, granular, tricolporate, reticulate, muri broad, flat, thick; lumina small irregularly shaped, colpi deeply intruding, and 33.2 μm in size. The pollen-ovule ratio is 1,643.2:1. The flowers begin nectar secretion along with anther dehiscence. The nectar secretion occurs in minute amount which has accumulated at the ovary base and on the yellow part of petals and it glitters against sunlight. It is quite prominent during the entire period of stigma receptivity. A flower produces 0.4 ± 0.08 μl (Range 0.3-0.5) of nectar with a sugar concentration of 38%. The sugar types included glucose and fructose and sucrose with the first as dominant. The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is 12% in spontaneous autogamy, 33.33% in hand-pollinated autogamy, 40% in geitonogamy, 68% in xenogamy and 55% in open pollination.

The flowers were foraged consistently during day time from 07^{00}-17^{00} h by insects. The insects included *Halictus* sp., *Chrysomya megacephala, Eristalinus arvorum, Rhyncomya* sp., an unidentified fly, *Polistes humilis* and *Catopsilia pyranthe*. Individuals of each fly species were numerous on each tree. Bees were the exclusive pollen feeders. They also collected nectar throughout the flower life depending on the availability. All other insects collected nectar only. Each forager species made 11.5 to 17.5% of foraging visits (Fig. 8).

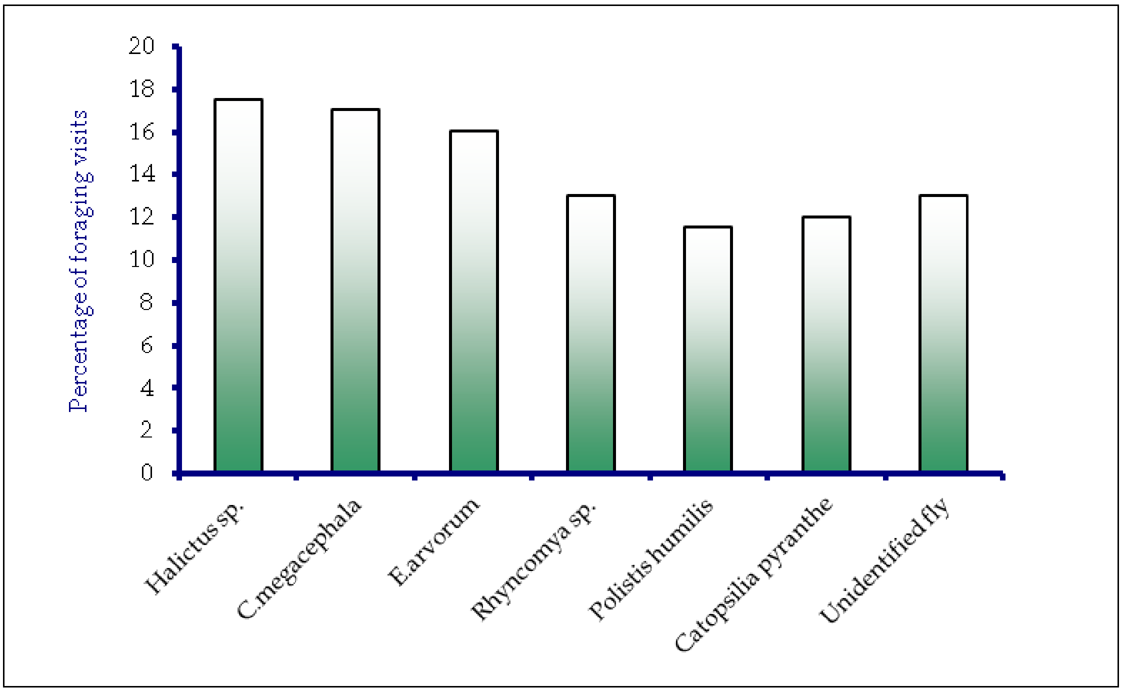

Figure 8: Percentage of foraging activity of insect organisms on *Avicennia marina* species.

All the insects probed the flowers in upright position to collect the forage. *C. pyranthe* landed on the petals, stretched its proboscis to collect nectar aliquots on the petals and at the flower base. In this process, all the insects invariably touched the anthers and the stigma; the ventral side of all insects was found with pollen. Further, the body washings of the all insect species revealed the presence of pollen; the average number of pollen grains per insect for each species varied from 63.1 to 227.4. As the nectar is secreted in minute amount, the insects made multiple visits to most of the flowers on a tree and moved frequently between trees to collect nectar. Such a foraging behaviour was considered to effect self- and cross-pollination.

Pollinated and fertilized flowers initiate fruit development immediately and take about a month to produce mature fruits. In each fertilized fruit, only one ovule produces seed. Fruit is a one-seeded leathery grayish green capsule with persistent reddish brown calyx; 30-35 mm long, 25 mm wide, abruptly narrowed to a short beak and hairy throughout. Seed produces light green, hypocotyls, which completely occupies the fruit cavity. An inflorescence produces 6.88 ± 2.96 fruits (Range 3-20). The Rose-ringed Parakeet, *Psittacula krameri* was found to feed on the hypocotyl by damaging the fruit pericarp; the percentage of damaged fruits is 4%. The fruit together with hypocotyl falls off the maternal parent, it settles in the substratum immediately at low tide period, it floats in water and disperses by tidal currents at high tide period until settled somewhere in the soil. The radicle side of hypocotyl penetrates the soil and produces root system while plumule side produces new leaves and subsequent aerial system. The fruit pericarp detaches and disintegrates when plumular leaves are produced.

Avicennia officinalis (Avicenniaceae). It is a tall evergreen tree with irregular spreading branches giving crowned globose appearance. It grows to a height of more than 15-20 m. The summer showers or early monsoon rains trigger flowering response. The rains vary in their occurrence and intensity even over a short distance; consequently the flowering period varies from place to place. The flowering season extends until August. The flowering density is almost uniform throughout the flowering season. Inflorescence is a terminal or axillary trichotomous panicle; each panicle produces 32 ± 11 flowers which anthese over a period of 16-25 days. Flowers are small, orange-yellow, ten mm long, ten mm wide, cup-shaped, have foetid smell, bisexual, slightly zygomorphic and oriented erect or partly horizontal. Calyx is shorter and has four ovate light green sepals with hairs on the outer surface; it is persistent. The corolla has four thick, light to orange yellow glabrous petals forming a short tube at the base; the petal margins are dull white. The adaxial petal is the broadest and shallowly bilobed. Stamens are four, epipetalous, three mm long and inserted basally on corolla. The anthers are basifixed, exserted, introrse and arranged alternate to petals. The ovary is very small (seven mm long), conspicuously hairy, bicarpellary syncarpous with four imperfect locules and each locule contains one pendulous ovule. It is terminated with a two mm long glabrous style tapered to the unequal bifid hairy stigma; both the style and stigma are light yellow throughout the flower life. The entire female structure is over-arched by stamens above. The style is bent, situated below the adaxial corolla lobe but not in the centre of the flower.

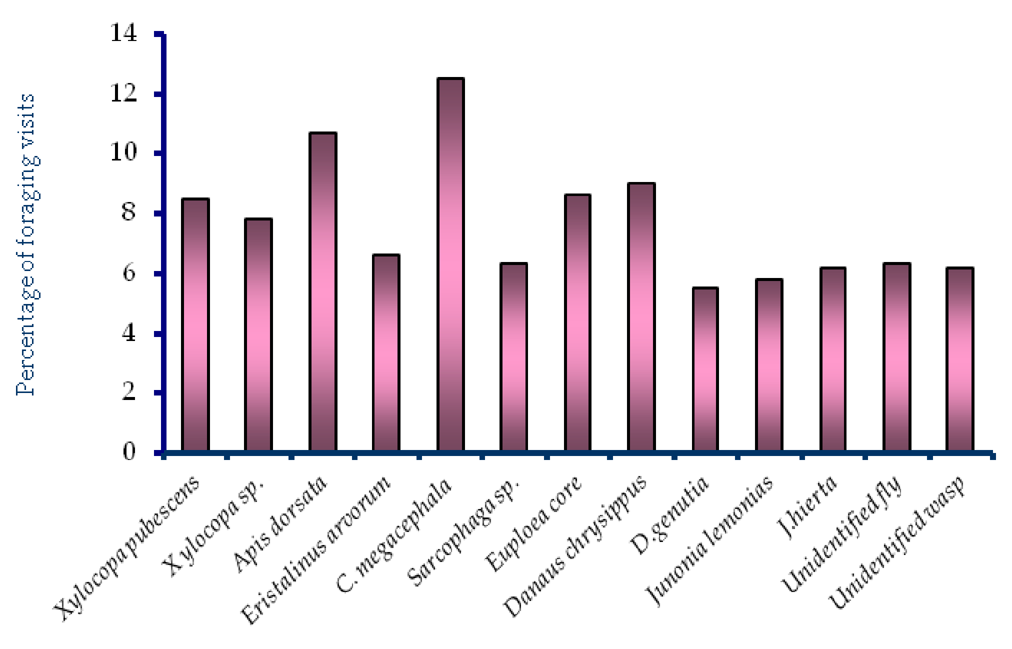

Figure 9: Percentage of foraging visits of insects on *Avicennia officinalis*.

The mature buds open throughout the day with most mature buds opening during 08^{00}-11^{00} h. The petals slowly open and take two-three hours for complete opening to expose the stamens and stigma. The petals emit foetid smell at anthesis. The stamens bend inward overarching the stigma at anthesis and dehisce 0.5 h after anthesis. All the four anthers dehisce at the same time by longitudinal slits. Gradually, they become erect and bend backwards; to achieve this, they take three days. Then, the anthers became dark brown and petals turn light orange. Gradually, the bent stigma grows, becomes erect and is bifid on the morning of the third day. The bifid condition of stigma is an indication of beginning of stigma receptivity and it remains receptive until the fifth day. A flower lasts for seven days. The petals, stamens and stigma drop off while the calyx is persistent. The pollen production per anther is 2,444 ± 202.4 (Range 2078-2604) and per flower is 8,837. The pollen grains are light yellow, granular, tricolporate, reticulate, muri broad, flat, thick; lumina small irregularly shaped, colpi deeply intruding, and 33.2 µm in size. The pollen-ovule ratio is 2,209.3:1. The flowers begin nectar secretion along with anther dehiscence. The nectar secretion occurs in minute amount of the yellow part of petals and it glitters against sunlight. It is quite prominent during the entire period of stigma receptivity. A flower produces 0.65 ± 0.09 (Range 0.5-0.8) µl of nectar with a sugar concentration of 39.75 ± 1.89% (Range 36-43%). The sugar types included sucrose, glucose and fructose with the first as dominant. The nectar amino acids included aspartic acid, cysteine, alanine, arginine, serine, cystine, proline, lysine, glycine, glutamic acid, threonine and histidine. All of these are prominent except lysine, glycine and proline. The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is 21.42% in spontaneous autogamy, 42.85% in hand-pollinated autogamy, 63.33% in geitonogamy, 67.85% in xenogamy and 58.13% in open pollination.

The flowers were foraged consistently during day time from 07^{00}-17^{00} h by insects. The insects included *Apis dorsata, Xylocopa pubescens, Xylocopa* sp., *Eristalinus arvorum, Chrysomya megacephala, Sarcophaga* sp., *Euploea core, Danaus chrysippus, D. genutia, Junonia lemonias, J. hierta*, a fly and a wasp (unidentified). Individuals of each fly species were numerous at the flowers. *A. dorsata* was the only pollen feeder. It also collected nectar throughout the flower life depending on the availability. All other insects collected nectar only. Each forager species made 5.5 to 12.5% of foraging visits (Fig. 9). All the insects probed the flowers in upright position to collect the forage. In case of *Xylocopa* bees, they made audible buzzes while collecting nectar aliquots from the petals. The butterflies landed on the petals, stretched their proboscis to collect nectar aliquots on the petals. In this process, all the insects invariably touched the anthers and the stigma; the ventral side of all insects was found with pollen. Further, the body washings of the all insect species revealed the presence of pollen; the average number of pollen grains per insect for each species varied from 73 to 550.2. As the nectar is secreted in minute amount, the insects made multiple visits to most of the flowers on a tree and moved frequently between trees to collect nectar. Such a foraging behaviour was considered to effect self- and cross-pollination.

Pollinated and fertilized flowers initiate fruit development immediately and take about a month to produce mature fruits. In fertilized flowers, only one ovule produces seed. Fruit is a one-seeded leathery capsule with persistent reddish brown calyx; 30 mm long, 25 mm wide, abruptly narrowed to a short beak and hairy throughout. Seed produces light green, hypocotyl which completely occupies the fruit cavity. The fruit pericarp dehisces when the hypocotyl is ready for dispersal and when the latter is still on the mother tree. The entire dehisced fruit with hypocotyl inside falls off from the mother tree. The fruit pericarp together with hypocotyl settles in the substratum immediately at low tide period; it floats in water and disperses by tidal current at high tide period until settled in the soil. The radicle side of hypocotyl penetrates the soil and produces root system while plumule side produces new leaves and subsequent aerial system. The fruit pericarp detaches and disintegrates when plumular leaves are produced.

Aegiceras corniculatum (Myrsinaceae). It is a small evergreen shrubby tree with spreading diffused branches, growing to a height of four-five m. It flowers mainly from second week of February to second week of April; sporadic flowering occurs outside this period, especially during rainy season. The plant species such as *Bruguiera gymnorrhiza* (year-long bloomer), *B. cylindrica* (seasonal bloomer, September-March), *Ceriops decandra* (year-long bloomer) and *Excoecaria agallocha* (seasonal bloomer, July-August) occur in association with *A. corniculatum*. In *A. corniculatum*, an individual tree flowers for 22-25 days. Inflorescence is a simple umbel which arises in the shoot apex or in auxillary branches. An inflorescence produces 21.53 ± 4.42 flowers (Range 14-36). There is considerable synchrony of flower development in one umbel and in this state, the flowers appear in ball-like clusters. The umbels open in acropetal succession.

Flowers are pedicellate, pointed in bud condition, 17 mm long, seven mm wide, white, cup-shaped, fragrant, bisexual and zygomorphic. Sepals are five, small, five mm long, creamy white, twisted, free, coriaceous, round apex and persistent. Petals are five, free, five mm long, white, twisted to the left, slightly fused at base to form short tube of five-six mm length, mouth with a dense weft of hairs and capitate hairs at the base. Stamens are five, opposite to petals, epipetalous, adnate to the base of the corolla tube, filament creamy white, five mm long, base united to form a tube with a ring of internal and external hairs at the level of the mouth of the corolla tube. The anthers are bilobed, two mm long, inserted, sagittate, introrse and medifixed. Ovary is superior, conical, eight mm long, single-loculed with 35 ovules arranged on free central placentation in one chamber. Style is terminal, elongated, creamy white with dark brown dots, three mm long and extends beyond the mouth of the corolla tube. It has pedicellate glands at the base of the style and a nectariferous area at the base of the ovary, glabrous, soft, gradually tapering; stigma is apiculate.

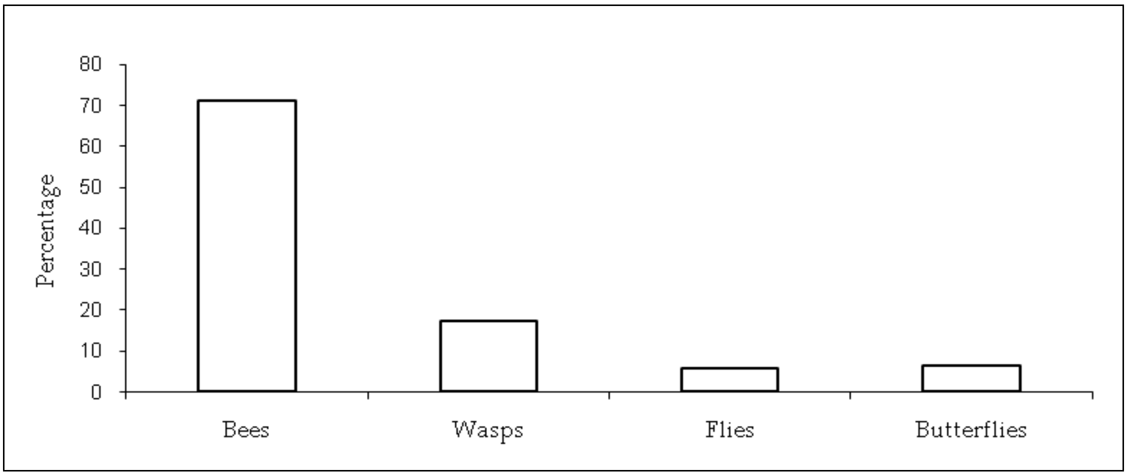

Figure 10: Percentage of foraging visits of insect categories on *Aegiceras corniculatum*.

The mature buds open at 06^{00} h by slightly unfolding the petals. Gradually, the erect petals take horizontal position and finally reflex backwards exposing stamens and the single style; this entire process takes place in a time span of three hours. The anthers enclose the style and stigma and are bend towards it; both parts attain almost the same height. Anther dehiscence occurs one hour after anthesis by longitudinal slits. The pollen output per anther is 15,221 ± 1528.09 (Range 12,864-18,336) and per flower is 76,105. Pollen grains are spheroidal, tricolporate with distinct annulus, dull white, exine smooth, thick and 33.2 µm in size. The pollen-ovule ratio is 2,174:1. The pollen protein content per anther is 5.6 µg and per flower is 28 µg. Pollen grains are viable for 3.5 days. The stigma attains receptivity almost at the time of anther dehiscence and continues up to the evening of the third day. A flower produces 4.08 ± 0.6 µl (Range 2.2-5) of nectar from the ring of tissue at the base of the ovary. The nectar sugar concentration is 36.46 ± 3.7% (Range 32-40%) and the common sugars include fructose and glucose with the former relatively more dominant. There is no significant correlation between nectar volume and sugar concentration ($r = 0.462$ at $p > 0.05$ significance level). The total sugar content in the nectar of a flower is 1.73 ± 0.39 mg (Range 0.76-2.63). The nectar contains 13 amino acids which include tyrosine, glycine, proline, lysine, aspartic acid, glutamic acid, serine, cysteine, cystine, alanine, threonine, arginine and histidine. The flowers not pollinated fall off on the fifth day. In pollinated flowers, the petals and stamens drop off on the fourth day while the fruit is in a growing stage. The sepals and style are persistent, and remain on the plant even after the shedding of propagule. Floral bud abortion is 2%. The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is 7% in wind-pollinated flowers, 15.8% in spontaneous autogamy, 40.0% in insect-assisted pollination and 54.6% in open pollination. Fruit set per inflorescence in open pollination is 13.96 ± 5.05 (Range 5-24).

The flowers are unspecialized and the stamens and style become exposed when the petals reflex backward. They were foraged during day time from 06^{00}-17^{00} h. The foragers included bees (*Apis dorsata, A. cerana, A. florea, Amegilla* sp., *Nomia* sp., *Megachile* sp., *Xylocopa pubescens, X. latipes, Xylocopa* sp.), wasps (*Delta campaniforme, Polistes humilis* and *Odynerus* sp.), a fly species, *Chrysomya megacephala* and butterflies (*Catopsilia pomona, Euploea core* and a Hesperiid). All these started their forage collection from 06^{00} h and continued until 17^{00} h with varying number of foraging visits at each hour. Bees collected both pollen and nectar throughout the day and accordingly the number of foraging visits were found to be nearly consistent. In the afternoon hours, bees were found to collect primarily pollen. Wasps, the fly and butterflies collected only nectar. They made a number of foraging visits during forenoon hours and gradually their visits were reduced towards the end of the day. Of the total number of foraging visits of insects, bees made 71%, wasps 17.1%, the fly 5.5% and butterflies 6.4% (Fig. 10). Further, *A. dorsata* and *Xylocopa* species individually made higher percentage of foraging visits. All these insects while probing the flower for nectar and/or pollen invariably contacted the style, stigma and stamens. These insects while probing for nectar contacted the sex organs with their ventral and/or dorsal side. In case of butterflies, the proboscis usually contacted the sex organs; the head and wings rarely contacted the floral sex organs. Bees while collecting pollen always made contact with their ventral side. Body washings of insects revealed the presence of pollen grains; the mean number varied from 51.9 to 1552. Bees were found to carry more number of pollen grains than all other categories of insects; the number of pollen grains found appeared to be related to the size of the bee species. Wasps with their smooth bodies were found to carry a small number of pollen grains. The fly was found to carry relatively more number of pollen grains than the wasps. Butterflies were found to transfer pollen grains principally through their proboscis; the proboscides were found with an average number of 93.3-117.3 pollen grains. Bees were also found to forage for pollen and nectar of *Bruguiera gymnorrhiza* occasionally. *Nomia* bees and *Odynerus* wasps also collected forage from *Ceriops decandra* occasionally. High winds prevailing in the plant area enabled the medifixed anthers with free movement to release light, dry and powdery pollen grains into the air easily.

Pollinated and fertilized flowers initiate fruit development immediately and take 30-35 days to produce mature fruits. Of the 35 ovules, only one ovule enlarges and the others remain undeveloped. The developing ovule displaces the placenta laterally while the young seed elongates. Fruit is an elongated, one-seeded capsule, light green to pink, 65 ± 5 mm long (Range 50-80), completely curved with pointed apex, persistent calyx and filled at maturity by the embryo with extended radicle and attached laterally by a long funicle-like structure. Seed coat is hard, brown, hairy with placental remains attached to mature seed coat and at the hypocotyl tip. Hypocotyl comes out of the seed coat but it does not pierce the pericarp. Mature fruit with well developed hypocotyl inside hangs downwards. They are water-buoyant and dispersed by tidal waters. After detachment from the mother plant, fruit pericarp dehisces longitudinally by absorbing water. The hypocotyl is not water-buoyant without fruit pericarp. In dehisced fruit, the green hypocotyl is exposed and produces a new plant when settled in a suitable substratum. The radicle side of hypocotyl penetrates the soil and produces root system while plumule side produces new leaves and subsequent aerial system.

Aegialitis rotundifolia (Plumbaginaceae). It is a soft-wooded evergreen shrub species, growing to three m height with a basally swollen fluted axis. It occurs in association with *Ceriops decandra*, *C. tagal*, *Bruguiera gymnorrhiza* and *Excoecaria agallocha*. Its leaves excrete salt which in turn gets crystallized on the leaf surface on sunny days. The flowering occurs from second week of February to third week of April. An individual tree flowers for 20-22 days. The flowers are produced in terminal, irregular one-sided cymes with pairs of opposite linear bracteoles. An inflorescence produces 6.2 ± 1.6 flowers (Range 4-10) over a period of six or seven days. Flowers are pedicellate, small, 18 mm long, 11 mm wide, white, cup-shaped, odourless, bisexual and zygomorphic. Sepals are five, small, nine mm long, green, united basally and free apically, glabrous, coriaceous, valvate and persistent. Petals are five, alternate to sepals, 12 mm long, white, herbaceous, free above with bluntly rounded lobes, fused basally to form a corolla tube of three mm long. Stamens are five, 12 mm long, free, inserted on the corolla tube alternately with the petals. The filaments are seven mm long, whitish, glabrous, slender, forming short hollow tube with a ring of internal and external hairs at the level of the mouth of the corolla tube. The anthers are bilobed, two mm long, inserted, sagittate, introrse and basifixed. The ovary is superior, oblong, one-chambered with a single basally attached anatropous ovule. It has five grooves each extending into a lobe which in turn extending into a free style. The styles are five, free, white, each style ten mm long with an extended oblique peltate stigma initially facing inward and later facing outward.

The mature buds open at 07^{00}-09^{30} h by slightly unfolding a single petal first followed by the second petal and other petals within two hours. Petals reflex backward partially exposing the stamens and the styles. The styles and stigmas stand slightly below the height of the anthers, face inward at anthesis and diverge gradually moving away from the anthers but reaching closer to the petals. The anthers dehisce by longitudinal slits along with anthesis. The pollen output per anther is 288.6 ± 40.27 (Range 240-372) and per flower is 1443. Pollen grains are large, spheroidal, tricolporate characterized by prominent central wart-like sculptures, light yellow, exine rough, thick and 119.52 ± 10.49 µm in size. The pollen-ovule ratio is 1443:1. The pollen protein content per anther is 6.08 µg and per flower is 30.04 µg. Pollen grains are viable for 2.5 days. The stigma attains receptivity two hours after anthesis and continues up to the evening of the third day. A flower produces 6.50 ± 0.8 (Range 5-7.8) µl of nectar at the flower base by the time of anthesis. The nectar sugar concentration is 46.2 ± 5.4% (Range 36-53%) and the common sugars include fructose and glucose which occur in almost equal amounts. There is no significant correlation between nectar volume and sugar concentration ($r = -0.386$ at $p > 0.05$ significance level). The total sugar content in the nectar of a flower is 3.60 ± 0.49 (Range 3.07-4.64) mg. The nectar contains 16 amino acids which include tyrosine, glycine, lysine, aspartic acid, glutamic acid, serine, cysteine, cystine, alanine, threonine, arginine, phenylalanine, proline, tryptophan, valine and histidine. The corolla gradually turns from white to dark red from day one to day four. The dark red corolla together with stamens and styles remain in place for two to three weeks during which the calyx bulges due to growing fruit inside. The calyx is persistent and inseparable from fruit. The flowers not pollinated fall off on the fourth day. The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is 9% in wind-pollinated flowers, 25% in spontaneous autogamy, 47% in insect-assisted pollination and 60% in open pollination. Fruit set per inflorescence in open pollination is 2.62 ± 1.41 (Range 1-7).

The flowers are unspecialized; the stamens and styles to become exposed when the petals reflex backward partially. They were foraged during day time from 07^{00}-16^{00} h. The foragers were exclusively bees which included honey bees, *Apis dorsata, A. cerana, A. florea* and the Stingless bee, *Ceratina simillima*. All the bees collected pollen and nectar in the same and/or in a different foraging visit. Their foraging activity was primarily concentrated in the forenoon period and gradually decreased towards the end of the day. Of the total number of foraging visits of insects, *A. dorsata* and *A. florea* together made 64.5% while the other two bee species made the remaining percentage of visits (Fig. 11). All the bees while probing the flower for nectar and/or pollen invariably contacted the styles and stamens. The *Apis* bees were also found to forage for pollen and nectar of *Bruguiera gymnorrhiza* while *C. simillima* collected pollen and nectar of *Ceriops decandra*. High winds prevailing in the plant area enabled the anthers to release light, dry and granular pollen grains into the air easily.

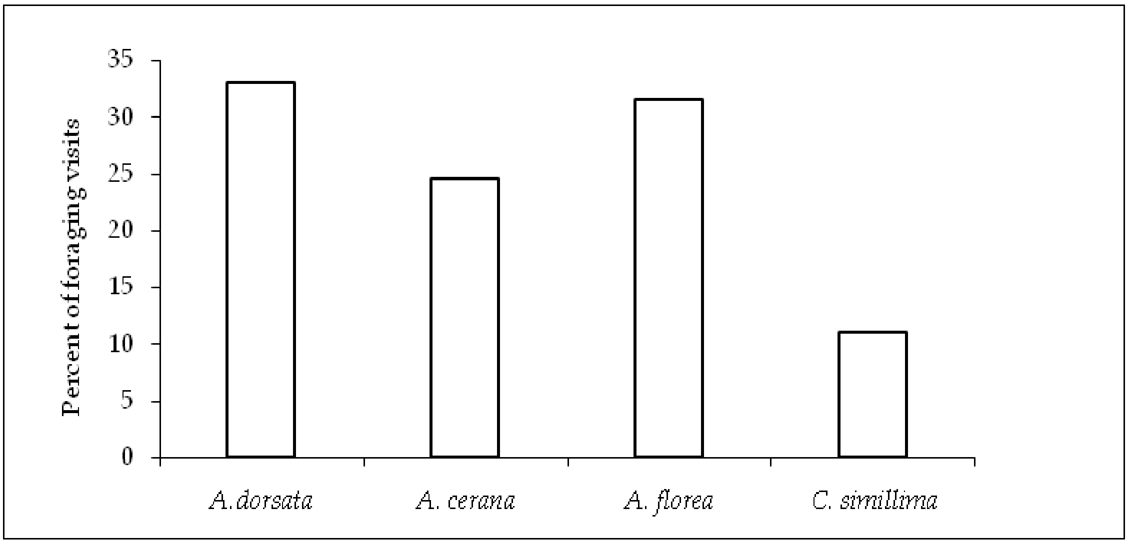

Figure 11: Percentage of foraging visits of bees on *Aegialitis rotundifolia.*

Pollinated and fertilized flowers initiate fruit development immediately and take 30-45 days to produce mature fruits. Fruit is an elongated, bluntly pointed one-seeded capsule, light green to brown, 72 ± 4 mm (Range 65-83) long, enveloped basally by persistent calyx, funicular tube attached to seed and enlarging hypocotyl which protrudes from the seed coat but not the pericarp. Seed coat is hard, brown, embryo elongated with an extended hypocotyl. Mature fruit with well developed hypocotyl stands upright. Fruit pericarp is thin but thickened somewhat distally. It is water-buoyant and dispersed by tidal waters. It detaches along with the quadrangular calyx. The hypocotyls settle at the mother plant if the site is not inundated due to tidal water and float in tidal waters, especially during high tide periods. In fruits that float in tidal waters, the pericarp becomes soft and ruptures longitudinally to expose the hypocotyl to saline water. The hypocotyls devoid of fruit pericarp did not float while those with it floated. Such hypocotyls float until they find suitable substratum which is sticky and silty mud. The radicle side of hypocotyl penetrates the soil and produces root system while plumule side produces new leaves and subsequent aerial system. In the study area, very few propagules were found to settle, establish and produce new plants.

DISCUSSION

Ceriops tagal **and** ***Ceriops decandra***. It is strictly a winter bloomer whereas its closely related species, *C. decandra* is a year-long bloomer with alternate flowering and fruiting phases (Solomon Raju et al., 2006). Aksornkoae et al. (1992) reported that the occurrence of the two species at the same site is rare; we also found similar situation at the Krishna mangrove forests. The distribution of *C. tagal* in this forest indicates that it has a distinct seaward zonation and prefers well drained high saline soils, suggesting that the species is a salt tolerant mangrove with the competitive ability to grow in highly saline and partly inundated locations (Aziz and Khan, 2001). The site is flooded with water only during high tides and is well drained during low tides indicating that *C. tagal* is a higher inter-tidal mangrove specialist and the plants occurring in such sites are inundated about twenty times a month (Duke et al., 1998). Further, in such sites, rainfalls make no differences and hence, the salt content of the soil remains high and approximately uniform throughout the year. Duke et al. (1998) reported that *Excoecaria agallocha* becomes more common in the absence of *C. tagal* in such sites. At the study site, a few trees of *C. tagal* occur with some naked habitat and *E. agallocha* grows here and there in its association. On the contrary, *C. decandra* is not a strict seaward mangrove plant and it occurs commonly even in areas of tidal zone far away from sea shore (Tomlinson, 1986). Field studies here showed that *C. tagal* is absent in Godavari mangrove forest, while a few trees still survive in Krishna mangrove forests. Therefore, *C. tagal* being a seaward mangrove, it is highly unsuccessful to establish a good population size, while its sister species, *C. decandra*, with flexibility to survive in tidal zones even far away from the seashore, is highly successful to build up its populations to the extent of becoming a common constituent of mangrove forests.

In *C. tagal*, the floral characteristics such as white flowers, strong fragrance, complex petal-stamen configuration and production of moderate amount of nectar suggest an elaborate and specialized floral mechanism. The petals require an external delicate touch for the explosive release of stamens. The helically coiled hairs at the lower margins of the petals help to propagate explosive pollen release effectively (Juncosa and Tomlinson, 1987). The petal clavate appendages of petals in *C. tagal* lack hydathodes and abundant xylem which are characteristically present and have a role in flower function under extreme water pressure deficits during the day in *C. decandra* (Juncosa and Tomlinson, 1987). Such a state may make appendages very light and provide necessary trigger for petal explosion by delicate touch by the forager in *C. tagal*. Explosive pollination mechanism has also been reported in *Bruguiera* species for which the flower tripping agents are birds and butterflies (Tomlinson et al., 1979; Ge et al., 2003).

Tomlinson (1986) reported night-flying insects, especially moths, as probable pollinators. Meeuse and Morris (1984) described the characteristics of moth flowers which include flower opening in the evening, display of overwhelming fragrance at that time, light flower colour, absence of landing platform, fringed petals for guidance, visual and olfactory nectar-guides, long and narrow corolla tube, abundance of nectar and short-tongued visitors. Baker and Baker (1983) reported that hawk moth flowers produce sucrose-rich or dominant nectar with low sugar concentration. Cruden et al. (1983) reported that small moth flowers produce relatively small volume of nectar with small amount of sugar. They also stated that moth flowers initiate nectar secretion one-three hours or even ten or more hours prior to the activity period of moths. In the *C. tagal,* nectar guides and tubular corolla are lacking, the nectar secretion begins an hour after anthesis; it is in moderate volume, hexose-rich with high sugar concentration. These characters together with the shallow nature of flowers are suitable for foraging by short-tongued bees and flies (Baker and Baker, 1983; Cruden et al., 1983). In this study, there was no foraging activity of hawk moths or settling moths at the flowers after anthesis til late evening (up to 22^{00} h) and the absence of moths could be due to non-availability of nectar at anthesis and reduced opportunities for food and breeding opportunities in harsh mangrove habitats. *C. tagal* with a few trees and a few numbers of flowers per unit of time per tree does not constitute a potential nectar station for moths. Further, adult moths do not survive for longer periods and in particular, hawk moths may survive for a period exceeding a month (Opler, 1983). Within that life span, the availability of nectar in the habitat is crucial and since *C. tagal* is unable to attract and supply its nectar requirements, the moths might have disappeared or migrated to other reliable food-rich habitats. *Apis* bees and *Chrysomya* flies make up day-time foragers for *C. tagal*. With a small number of trees and again each tree with a small number of flowers per day, *C. tagal* is not a potential pollen and nectar source for honey bees. Yao et al. (2006) also reported that this plant species is a minor pollen and nectar source for honey bees. In the study site, *Aegiceras* and *Bruguiera* flowers attract honey bees and the latter were found concentrating on these species. *Chrysomya* flies frequent the flowers of *C. tagal* daily effecting pollination but they have limited pollen transport capacity; this however, is compensated by their numbers and could bring about substantial geitonogamy and xenogamy (Faegri and van der Pijl, 1979). The petal colour change may act as a nectar guide for the flies to visit the flowers for several days. The close proximity between trees of *C. tagal* at the study site also facilitates xenogamy. The fly is present throughout the year unlike periodic bees and moths; but its presence depends on local opportunities for breeding sites (Faegri and van der Pijl, 1979). The butterfly is an occasional forager and has no role in pollination. Our observations suggest that *Chrysomya* is the primary and consistent pollinator, while honey bees are secondary and occasional pollinators. Petal explosion also occurs sporadically in nature due to wind action and this is evident in bagging experiment in which there is a negligible fruit set and also in natural conditions to some extent. The role of wind in tripping explosive pollination has also been reported in *Hyptis suaveolens* (Lamiaceae) (Solomon Raju, 1989) and *Shorea robusta* (Dipterocarpaceae) (Aluri et al., 2004). Therefore, petal explosion and subsequent pollination events are primarily vector-dependent and inadequate numbers or non-availability of pollinators are bound to result in reduced or no fruit set in *C. tagal*.

Less is known about the importance of amino acids in floral nectars to foragers. Amino acids are the second most abundant class of the compounds after sugars to be found in nectar (Gardener and Gillman, 2002). Their concentrations in nectars are considerably lower than sugar concentrations. But even the slightest concentrations are important nutritionally and also contribute to the "taste" of the nectar (Baker and Baker, 1983). Honey bees respond to differences in amino acid concentration and detect amino acids. They prefer certain amino acids and their presence makes nectar more attractive (Dress et al., 1997). Tyrosine is not an essential amino acid, but may be important in the formation of sclerotin (Gardener and Gillman, 2002). Phenylalanine is a precursor of specific honey aroma component, phenyl-ethanol (Thawley, 1969), iso-leucine is required for rapid breeding (Slansky and Feeny, 1977). Flies also prefer amino acids in nectar. In the flies, *Boettcherisca peregrine* and *Phormia regina*, proline stimulates salt receptor cells, methionine and valine stimulate sugar receptors, methionine also elicits a feeding response from flies and glycine and serine invoke an extension of the proboscis (Shiraishi and Kuwabara, 1970; Goldrich, 1973). *C. tagal* flowers with a mix of floral characteristics of moth, bee and fly flowers contain conventional protein building amino acids such as tyrosine, glycine, methionine, proline, lysine, aspartic acid, glutamic acid, serine, cysteine, alanine, threonine and arginine but are devoid of other protein-building amino acids such as phenylalanine, valine, leucine, iso-leucine, tryptophan and histidine. The nectar provides an instant supply of methionine, lysine and arginine for honey bees and flies; moths if occur in the habitat also make use of this nectar for protein building. The presence of several amino acids in this nectar source stimulates feeding and may also be an important source for flower foragers.

C. decandra as a common species in most of the regions and its distribution range may have achieved distinct out-crossing rates and accordingly, it has been reported to be an out-crosser (Solomon Raju et al., 2006). On the contrary, *C. tagal* with a small number of individuals at the study site can produce offspring with mixed mating system only if it fails to attract potential and adequate pollinators. In line with this, we have found that it possesses such a system to produce fruit set even through autogamy. But, autogamy is negligible as realized in bagging experiment. In *Kandelia candel* and also in a crypto-viviparous species, *Avicennia marina*, negligible self-pollination has been reported in bagging experiments (Sun et al., 1998; Clarke and Myerscough, 1991). The fruit set in *C. tagal* is through geitonogamy and xenogamy. The long flower life and stigma receptivity and high pollen-ovule ratio (Cruden, 1977) indicate that the plant is primarily out-crossing. However, the close proximity of the existing trees at the study site in the course of time could bring about genetic uniformity and if this happens, then the survival of this species becomes doubtful.

In *C. decandra*, the floral characteristics such as small white flowers lacking fragrance, simple stamen-petal configuration, short, thick filaments and production of trace amount of nectar suggest a simple floral mechanism. Juncosa and Tomlinson (1987) stated that the short basal hairs of petal edges have no evident function in *C. decandra*, but these hairs are well developed and help to propagate explosive pollen release effectively in fragrant flowers of *C. tagal*, in which the petal-stamen configuration is elaborate and specialized. Further, these authors reported that petal clavate appendages in *C. decandra* have abundant xylem elements with a significant reservoir of water and hydathodes at or near the termini; they have significance in flower function under extreme water pressure deficits during the day in mangrove swamps. Abundant xylem is absent in petal appendages in *C. tagal* and *K. candel*, which are pollinated at night or early in the morning (Juncosa and Tomlinson, 1987). Therefore, the abundant xylem and hydathodes and their function in petal appendages of *C. decandra* suggest that the latter is adapted for pollination during the day. Tomlinson (1986) suggested that wasps and flies are suitable for pollination in *C. decandra*. Juncosa and Tomlinson (1987) noted that trigonid bees and small insects visit *C. decandra* flowers. In the present study, bees while collecting pollen and nectar and wasps while collecting nectar pollinate *C. decandra* flowers consistently. Female bees of *Nomia* and worker bees of *Trigona* collect pollen voraciously for brood provisioning. Female wasps collect nectar after mating for the maturation of the eggs. The plant produces a small number of flowers daily and accordingly, the pollen and nectar available is also in small quantities. Further, the anthesis process is gradual and the accumulation of new flowers with pollen and nectar begins to appear from late morning. As a consequence, bees and wasps also delay their foraging activity and appear from late morning onwards and stop foraging in the early evening. As flower number and floral rewards are small at the plant level, both bees and wasps fly between individuals of *C. decandra* in quest of more forage and this foraging behaviour brings about cross-pollination. Hand-pollination tests showed that it is an obligate outcrosser. The protandry, long period of stigma receptivity and long flower life substantiate this; pollination by bees and wasps favours outcrossing (Tomlinson, 1986). The pollen recovered from the body washings of these foragers suggests that they effect pollination. Earlier reports on bees, flies, wasps or even other insects as pollinators (Tomlinson, 1986; Juncosa and Tomlinson, 1987) and the present study on bees and wasps as pollinators suggest that *C. decandra* is strictly entomophilous and utilizes different locally available insects as pollinators. The commonness of *C. decandra* at Coringa mangrove forest provides ample opportunities for effective outcrossing and the genetic variation thus achieved would permit the species to survive well in the harsh mangrove environment.

In *C. tagal*, the number of fruits per inflorescence varies from one to five, but one- and two-fruited inflorescences are most common. The fruited flowers produce only one seed against the actual number of six ovules as in *C. decandra* and all other viviparous species of Rhizophoraceae. This characteristic may permit these plants to save resources and use them to produce one-seeded viable fruits. Despite this effort by *C. tagal*, a few propagules lack green pigment and becoming entirely yellowish or yellowish on one side and purplish on another side. Such hypocotyls have been referred to as “albino” forms which also occur in *C. decandra* and *Bruguiera gymnorrhiza* (Solomon Raju et al., 2006; Allen and Duke, 2006). These propagules are non-viable, cannot photosynthesize and die after depleting reserves if settled in the habitat. The propagules that are green first and brownish purple later are healthy and grow to their actual size. The length of hypocotyls in *C. tagal* is almost double the length of hypocotyls in *C. decandra*. Both the species of *Ceriops* show a short period of attachment to the maternal plant (Solomon Raju et al., 2006) and this characteristic is not in agreement with the report of Bhosale and Mulik (1991) that the hypocotyls of true viviparous mangrove species remain attached to the mother plant for a full year. The hypocotyl grows upward in *C. decandra* in which flowers are sessile (Solomon Raju et al., 2006), while in *C. tagal*, it grows downward which seems to be because of stalked flowers and more weight of the hypocotyls. This is an important field characteristic feature to distinguish *C. tagal* from *C. decandra*. The downwardly hanging hypocotyl is also a characteristic of *Bruguiera, Rhizophora* and *Kandelia* species. In *C. tagal*, the cotyledonary yellow cylindrical collar emerges from the fruit about ten days prior to the detachment of the hypocotyl while this structure is entirely absent in *C. decandra* (Solomon Raju et al., 2006). The cotyledonary collar is a characteristic also in *Rhizophora* in which it is reddish brown and in *Kandelia* in which it is yellow and the hypocotyl is about double the length of *C. tagal* (Aksornkoae et al., 1992).

Fruit in *C. tagal* grows continuously and the seed also has no dormancy like in other mangrove species of Rhizophoraceae (Farnsworth and Farrant, 1998). This form of fruit growth and seed germination leading to the formation of hypocotyl while still attached to the mother plant represents "vivipary", the opposite of "ovipary" in which seed dormancy is the rule. The viviparous condition has been considered as an evolutionary loss of seed dormancy (Farnsworth and Farrant, 1997), however, it is an adaptive feature for the plant to overcome the harsh tidal environment for seedling establishment in the parental sites but it is not considered adaptive for dispersal either in time or space (Sun et al., 1998). The other adaptive values of vivipary include facilitation of early rooting (MacNae, 1968), buoyancy during sea dispersal (Rabinowitz, 1978), transfer of maternal nutrients to the hypocotyls (Pannier and Pannier, 1975), maintenance of embryonic osmotic equilibrium and establishment in coarse grained environments. On the other hand, vivipary incurs maternal costs to supply water and necessary nutrients. Numerous attached seedlings may constitute a substantial carbon sink to the maternal plant, a concentrated apparent resource for herbivores (Farnsworth and Farrant, 1998). *C. tagal* at the study site was found to produce 20 to 60 hypocotyls per tree and it is not known whether this small number could attract herbivores.

Kairo et al. (2001) reported that viviparous mangrove species use self-planting or stranding strategy for establishment depending on forest conditions tide and stability of the soils. The self-planting strategy dominates in undisturbed mangrove forests but stranding strategy is dominant in exploited and open or naked forests (Dahdouh-Guebas et al., 1998). *C tagal* with epigeal seed germination, elongated and pointed hypocotyls with straight curvature (Clarke et al., 2001) fall freely from the mother plant and plant themselves into the mud at the same site during low tide period. The hypocotyls if fallen during high tides float to another site for settlement. But, our field studies do not show settlement of hypocotyls away from the mother plants suggesting that *C. tagal* uses self-planting strategy only. This is further substantiated by McGuinness (1997), who also reported that the hypocotyls of *C. tagal* in Northern Australia dispersed to very short distances; only 9% moved more than three m from the parent tree. He also mentioned that within that short distances, a high percentage of them were either damaged or eaten by animals. *C. tagal* at the study site may also be experiencing the damage or consumption by animals, especially crabs as the latter have been reported to show high predation on hypocotyls in high inter-tidal areas (Duke et al., 1998). Some of the fallen hypocotyls settled at the mother plants showed signs of weathering. Therefore, the study suggests that *C. tagal* though occurring in undisturbed and human-free site, is almost unable to add new plants and the presence of only a few individuals at the site attests this contention. The work reported in this paper is important for initiating studies on the genetic structure of *C. tagal* population. The genetic marker analysis helps to understand the variability within and between populations. Introduction of *C. tagal* from the mangrove forests of the Sundarbans, Andaman and Nicobar islands to this site would help to enhance gene flow in order to maintain the gene diversity and expansion of population size of *C. tagal* in Krishna mangrove forests.

The pollinated flowers initiate fruit growth and development immediately and produce mature fruits in about two months. Fruits are light green, ovoid, conical and blunt apically. They are distinct with five-lobed persistent calyx and produce only a single seed. The seed has no dormancy and it immediately produces spindle-shaped hypocotyls within three months, while still attached to the maternal parent. Then, it detaches from the residual fruit. The hypocotyl is slender, green, clearly ribbed, angular, sulcate, 15 cm long and broadened at the lower end. The short period of hypocotyl attachment to the maternal parent is a characteristic of cryptoviviparous species (Bhosale and Mulik, 1991). However, *C. tagal* shows this characteristic, being a true viviparous species (Selvam and Karunagaran, 2004). Further, the hypocotyl in *C. decandra* grows upright and is an important characteristic to distinguish it from *C. tagal* in which the hypocotyl grows downward (Kathiresan and Rajendran, 2003). In *C. decandra*, the hypocotyl is characteristically green and seems to have the potential to photosynthesize actively with water and necessary nutrients drawn from the parent tree. Viviparous reproduction allows hypocotyls to develop some salinity tolerance before being released from the parent tree. It provides a store of nutrients before the hypocotyls fall-off from the plant and helps in quick rooting in the muddy environment. The hypocotyl characteristics also help to develop buoyancy for distribution of the seedlings and structural stability to protect seedling from damage (Kathiresan, 2003). Therefore, vivipary could be an adaptive feature of the plant to overcome the harsh tidal environment for seedling establishment, especially in the parental sites. The small upright hypocotyls, when detached from the mother plant, float in water and settle at different places depending on the direction and extent of movement of tidal water. Such dispersal characterizes "Stranding Strategy".

Pollinator species of *C. tagal* and *C. decandra* need a special mentioning here. The fly pollinators have rich sources of breeding and feeding materials in the forests, but the breeding materials are subject to flooding during high tide periods and during rainy season. Bees and wasps use above ground plant materials such as live/dead branches/wood. But, these materials are usually collected by local people for use as fuel wood. The forage collection from plants occurring in windy areas is a difficult task for them and hence these foragers tend to collect the forage from the plants occurring landward. These foragers have no difficulty to collect forage from *C. decandra* as the latter is distributed principally landward. In case of *C. tagal*, it occurs seaward where wind blows at high speed which usually prevents or minimizes the foraging activity of honeybees. In consequence, the bees tend to collect the forage from the flowers covered by the branches and foliage where there is reduced wind speed.

The importance of bark of *C. decandra* in dyeing the cotton and fishing nets here is driving the fishermen to cut down trees indiscriminately and the tree cutting rate is further driven by the trade concept in some fishermen. Most of the areas where *C. decandra* occurs have been partly cleared for its bark and wood. The cleared areas show the stumps or the basal part of the cut trees and these areas are gradually being invaded by the oviparous weed species, *Excoecaria agallocha*. Further, some such areas are being used as cattle shelters by some villagers. Therefore, such areas cleared of *C. decandra* trees and the still existing stretches of *C. decandra* are an important consideration for the concerned forest authorities with regard to conservation, management and artificial regeneration of this species in naked and semi-naked mangrove habitats in order to provide a continuous supply of bark tannin for fishermen for treating their cotton fishing nets.

Rhizophora apiculata and ***Rhizophora mucronata*** are polyhaline, evergreen true viviparous tree species. The former species blooms throughout the year, but shows profuse flowering for two months during August-September while the latter species flowers for about six months with profuse to sparse flowering during August-September. Mulik and Bhosale (1989) reported that *R. mucronata* flowers throughout the year with intense flowering during September-November. The flowers are sessile and borne in pairs below the leafy cluster in *R. apiculata*, while they are pedicellate and borne in four-flowered cymes within the leafy cluster in *R. mucronata*. With foliage background, the flowers of *R. mucronata* are also quite prominent and may be more attractive to flower foragers when compared to those of *R. apiculata*. In the latter species, bee foraging activity has been found to be consistent during profuse flowering period than at other times of the year. This suggests that greater floral displays during profuse flowering period have an important role in attracting bee pollinators. The year-long flowering allows the plant to set fruit continuously and this may be a fail-safe strategy against pollination limitation and propagule predation.

In both the species of *Rhizophora*, the flowers exhibit certain adaptations for anemophily. The flowers are pendulous, point downward at maturity and situated below or within the leafy crown; this floral orientation is important to minimize interference of foliage for effective pollen dispersal by wind. The sepals diverge least, while the petals are glabrous, do not recurve or retain pollen grains in *R. apiculata*. But, in *R. mucronata* and in all other *Rhizophora* species, the petals are equipped with marginal hairs which have been shown to be promoters of anemophily by their hygroscopic movements (Tomlinson, 1986). Late morning anthesis, high pollen/ovule ratio, light powdery pollen, absence of an attractive colour and odour, absence of abundant pollinators and the presence of traces or minute quantity of nectar are some important characteristics for anemophily in the genus *Rhizophora* in general (Tomlinson, 1986) and in the studied species of *Rhizophora* in particular. Further, anther dehiscence and pollen release occur in mature bud in both *Rhizophora* species. In both, the late morning anthesis is another important characteristic for the effective dispersal of dry powdery pollen grains from the already dehiscent anthers due to moderate levels of temperature and humidity present at that time. The anthesis period noted in this study for *R. mucronata* does not agree with the report by Kondo et al. (1987) that the anthesis occurs in the afternoon from 15^{00} to 17^{00} h. These two plant species occur as pure stands mostly and are located in a windy environment along the creeks. This form of distribution may facilitate effective dispersal of pollen between individual trees and receptive sites of flowers receive wind-borne pollen. Kondo et al. (1987) reported that the pollen grains also have evolved characteristics for anemophily. Tomlinson et al. (1979) also experimentally proved that wind-borne pollen is abundant and hence anemophily is most efficient in such pure stands of other *Rhizophora* species. Although both *R. apiculata* and *R. mucronata* have several adaptations for anemophily, their stigma is not elaborated in the manner usual for wind-pollinated species to capture wind-borne pollen. Similar structure of the stigma has been reported in all other *Rhizophora* species (Tomlinson, 1986). The absence of marginal hairs on petals, lack of elaboration of stigma, absence of odour and presence of traces or minute quantity of nectar seem to be vestigial characteristics of entomophily. Kress (1974) also related these characteristics to entomophily in *R. mangle* and *R. stylosa*.

The flowers of *R. apiculata* attract the bees such as *Nomia*, *Trigona* and *Halictus*, while those of *R. mucronata* attract the bees such as *Nomia, Trigona, Halictus, Ceratina* and *Xylocopa*, and a moth species. Kondo et al. (1987) reported that *R. mucronata* is anemophilous, but is also pollinated by small insects like *Camponotus* sp., *Onychostylus pallidiolus* and a *Collembola*. In both the plant species, pollen is the principal reward since it is produced in huge amount. Further, the flowers are nectariferous but the nectar is secreted in traces or in minute quantity and hence has little importance for the foragers. The pollen and/or nectar feeding behaviour of these bees and the moth would contribute to pollination. But, their pollen feeding activity may considerably reduce the availability of airborne pollen and hence may affect the efficiency of anemophily. The copious production of pollen at flower level may compensate to some extent the pollen loss caused by pollen collecting bees. Further, thrips have been found in the floral buds of both *R. apiculata* and *R. mucronata*. Their presence in the buds indicates that egg deposition by female thrips takes place prior to bud formation; the eggs hatch, produce larvae or adult thrips by the time the buds mature. These thrips represent the suborder Terebrantia of the order Thysanoptera. The females of this group of thrips have an ovipositor with which they cut slits into plant tissue in order to insert their eggs, one egg per slit. With this ability, these thrips raise their offspring in floral buds. They are short-distance flyers, but windy areas extend their travel distance to a great extent. They feed on both nectar and pollen and in so doing contribute to both self- and cross-pollination, but the latter mode is more effective in areas where pure stands of *Rhizophora* species occur. The study suggests that the pollination mechanism in the studied *Rhizophora* species is originally entomophilous and is now being transformed to anemophily. This transitional stage of pollination mechanism is advantageous for these plant species to utilize both wind and insects as pollinating agents for the success of sexual reproduction. Tomlinson (1986) stated that animal pollination predominates in mangrove communities and in such a situation anemophily enables these species to escape the competition for insect pollinators and to set fruit in the total absence of insect pollinators.

Tomlinson (1986) reported that *Rhizophora* exhibits weak protandry and is self-compatible. He found that the isolated greenhouse plants set fruit. He has not mentioned what species of this genus are weakly protandrous and self-compatible. Coupland et al. (2006) reported that *Rhizophora* species show low rates of fruit set due to lack of autogamy. Ghosh et al. (2008) mentioned that a distinct trend for self-incompatibility exists in *Rhizophora*. In this study, *R. apiculata* and *R. mucronata* show anther dehiscence in mature bud and commencement of stigma receptivity soon after anthesis, suggesting that they are weakly protandrous. Tomlinson et al. (1979) reported that in these species, the petals and stamens fall off the flower on the day of anthesis leaving the central stigma alone in the empty flower. These authors also stated the stigma lacks any secretion or divergence of its two lobes. Kondo et al. (1987) reported that the stigma is two-lobed; each tip contains small papilla and secretes mucilage. In this study, it is found that the stigmatic lobes diverge soon after anthesis and since then it is in receptive stage to receive pollen. The stigmatic lobes are mucilaginous and have minute papilla, which may aid in capturing and retaining pollen readily.

Tomlinson et al. (1979) reported that in *Rhizophora stylosa*, an individual flower is generally incapable of self-pollination. Hand-pollination results suggest that *R. apiculata* and *R. mucronata* are capable of self-pollination either by gravitational fall of pollen or by wind driven pollen fall on the stigma. Further, these plants set fruit through allogamy, autogamy and geitonogamy (Kondo et al., 1987). The significantly low fruit set evidenced in open-pollinations is attributable to the intense pollen feeding activity of bees and also to the wastage of pollen due to wind activity. Coupland et al. (2006) also reported low fruit set to the extent of 13% in open-pollinations. The ability to set fruit through self- and cross-pollination is the characteristic of pioneering species in mangrove communities. *R. apiculata* and *R. mucronata* with this ability may become established as isolated individuals in new environments remote from parental source.

Like in *Bruguiera cylindrica*, the flowers are four-ovuled in both *Rhizophora* species, but only one ovule develops into mature seed in fertilized and fruited flowers. The production of one-seeded fruits may be due to maternal resource constraint or maternal regulation of seed set. Fruits mature within four to six weeks and are not enclosed by the persistent and expanded calyx at any stage. The calyx therefore seems to have no role in protecting the fruit. As the fruit is thick with hard pericarp, it does not require protection from the calyx. In both *R. apiculata* and *R. mucronata*, the single seed formed in the fruit is not dormant and germinates immediately to produce a cylindrical hypocotyls or seedling, while still on the maternal parent. The hypocotyl emerges out of seed and fruit pericarp and remains naked until it is detached from the maternal parent. This is a characteristic of “true viviparous” species (Tomlinson, 1986). While still attached to the maternal parent, the seedling develops chlorophyll and actively photosynthesizes; the parent tree supplies the water and necessary nutrients (Selvam and Karunagaran, 2004). The seedling hangs downward and detaches from the residual fruit at the collar end, leaving behind its cotyledons, and falls from the maternal parent. Christensen and Wium-Andersen (1977) speculated that in *R. apiculata*, the development from visible flower buds to mature propagules lasts about two years while Muniyandi (1986) reported that the propagule takes eight months to grow to full length after fertilization. But, in this study, it is found that this species produces mature seedlings from flower buds in a time span of about four months only. Muniyandi (1986) say that the seedlings of *R. mucronata* take 16 months to grow to full length after fertilization in *R. mucronata*. But, in this study, it is found that this species produces mature seedlings from flower buds in a time span of about four months only.

The fallen seedlings of *R. apiculata* and *R. mucronata* plant themselves into the mud in the vicinity of the maternal parent or are stranded and planted away from maternal parent. In the latter case, the seedlings sink after some period of dispersal and exhibit growth under water (Rabinowitz, 1978). The study shows that the seedlings disperse through self-planting and stranding strategies. Although dispersal distances have not been measured in this study, the available information shows that most viviparous seedlings do not disperse more than one km from their point of origin (Clarke, 1993), and in certain instances, as little as 65 m (Chan and Husin, 1985) or even three m (McGuinness, 1997). Davis (1940) found that *Rhizophora mangle* propagules carried by ocean currents could disperse at least 100 km. Smith (1987) reported seedling predation prior to and after detachment from the maternal parent in *R. apiculata* and *R. mucronata*. Monkeys and insects attack seedlings prior to their detachment, while crabs attack seedlings when they later fall in the mud or tidal water. Monkeys, insects and crabs collectively contribute to more than 83% of seedling predation. Bosire et al. (2005) reported that seedling predation is a mechanism to regulate individual species colonization, but in case of *R. mucronata*, the seedling predation is the least since they are very large and are not preferred by crabs. In this study, there is no seedling predation prior to detachment from the maternal parent in both the species of *Rhizophora*. Seedling predation by crabs may be there since different species of crabs have been found. The locals use crabs as a source of food and livelihood.

Bruguiera gymnorrhiza and ***Bruguiera cylindrica***. Both the species of *Bruguiera* are polyhaline, evergreen true viviparous tree species. *B. gymnorrhiza* is a year-long bloomer with concentrated flowering during April-June, while *B. cylindrica* shows flowering during September-March during which flowering level is almost uniform. On *B. gymnorrhiza*, pollinator activity is consistently intense during concentrated flowering period, while it is sporadic at other times of the year. On the contrary, pollinator activity is not consistent and also not intense at any point of time on *B. cylindrica*. This finding stated that pollinators preferentially visit plants with greater floral display. Therefore, fruit set is largely a function of pollinator activity during concentrated flowering period in *B. gymnorrhiza*. However, the characteristic of year-long or extended flowering in these two tree species is an important adaptation for additional fruit set; this characteristic would enable them to compensate the low fruit set rate which may result from pollinator limitation and also to compensate the loss of seedlings due to predation.

Tomlinson (1986) reported that *B. gymnorrhiza* represents the large, solitary-flowered group, while *B. cylindrica* represents the small, many-flowered group. The present study shows that it is true in case of *B. gymnorrhiza* but it is not so in *B. cylindrica* in which the flowers are small, but borne in three-flowered cymes. The flowers are pendulous in *B. gymnorrhiza*, while they are either horizontal or downward or slightly erect and located outward in the crown of leaves in *B. cylindrica*; these different orientations enable the pollinators to collect the forage comfortably. In both species, the anthesis period is the same, is confined to morning hours and quite appropriate for pollination by day-active pollinators. The field studies also indicate that their flowers receive visits exclusively from day-active pollinators. The flowers are red in *B. gymnorrhiza* while they are greenish-white in *B. cylindrica*. In both the species, the floral characteristics such as large or small flowers with concealed nectar, elaborate complex petal-stamen configuration, presence of basal clumps of hairs and marginal hairs suggest specialized explosive floral mechanism which is functional only when pollinating agents are involved. The same elaborate specialized mechanism is present in all other species of *Bruguiera* (Tomlinson et al., 1979). In *B. gymnorrhiza*, the petals are 14 and each petal encloses two stamens; this petal-stamen configuration requires multiple visits of pollinators for the explosion of all petals. In *B. cylindrica*, the flowers have only seven or eight petals with 14 or 16 stamens and closely spaced; this arrangement with reduced number of petals in relation to the small flower size is an important adaptation for the explosion of all petals of the flower and subsequent pollination in a single or two visits of the pollinator insects. Petal explosion is characteristically violent in *B. gymnorrhiza*, while it is non-violent in *B. cylindrica*. Further, similar explosive mechanism is present also in another mangrove Rhizophoraceae member, *Ceriops tagal*. Therefore, the floral mechanism in all the species of *Bruguiera* and in *C. tagal* is highly specialized and reflects an advanced state when compared to the simple floral mechanism that is present in *Ceriops decandra*, *Rhizophora* species and *Kandelia candel* - all belonging to mangrove Rhizophoraceae (Tomlinson, 1986; Juncosa and Tomlinson, 1987). Further, explosive pollination mechanism has been reported in a number of non-mangrove families such as Lamiaceae, Fabaceae, Onagraceae, Loranthaceae, Marantaceae, Urticaceae, Ericaceae, Fumariaceae, Musaceae, Cornaceae, Acanthaceae and Orchidaceae (Solomon Raju and Subba Reddi, 1995; 1996).

In *Bruguiera*, the explosive floral mechanism involves different functional aspects. At the time the flower opens, each petal pouch enclosing a pair of anthers includes loose pollen due to anther dehiscence during mature bud stage and stays in cocked position due to floral expansion and pressing of stamens against the interlocked margins of the petals. Marginal hairs appear to be important in holding the petals in the folded position. The petal explosion occurs due to release of tension by a slight touch at its hairy base by the probing pollinator during its vigorous search for nectar in the floral cup. During explosion, the petal unzips instantly, scattering a cloud of pollen, most or part of which falls on the pollinator. In each flower, individual petals work independently and this requires more than one visit of the same pollinator species or more than one pollinator species. This observation is substantiated by the occurrence of closed and open petals in each flower. The flowers with unexploded petals due to non-receipt of foraging visit(s) fall off without pollination or without ever-opening.

In *B. gymnorrhiza*, the following characters suggest adaptations for bird pollination: the flowers are recurved and typically point backwards into the crown of the tree; this facilitates an approach by a perching bird. Nectar is produced in abundance and held in the deep floral cup. The calyx is red, a colour attractive to birds. Large flowers with a heavy construction are suitable for a powerful pollinator like a bird (Faegri and van der Pijl, 1979). Azuma et al. (2002) examined *B. gymnorrhiza* flowers for scent characteristics and reported that the floral scent is lacking and the floral characteristics are indicative of bird pollination. Ghosh et al. (2008) also reported that the flowers are adapted to a range of flower visitors such as birds for pollination. Kondo et al. (1987) reported that this species is pollinated by honeyeaters, white eye and insects. Ge et al. (2003) mentioned that the flowers are pollinated by birds or butterflies. Solomon Raju (1989) reported that the flowers are exclusively pollinated by three species of passerine birds, *Nectarinia asiatica, N. zeylonica* and *Zosterops palpebrosus* at the Godavari mangrove site; then this site was ecologically healthy and there was little human interference. The present study finds that this site is now ecologically degraded and fragmented due to land use changes. With this present situation, *B. gymnorrhiza* is now pollinated exclusively by pollen and nectar collecting bees consisting of *Apis, Nomia* and *Halictus* genera. These bees trip the tensed petals to release the stamens which in turn eject a cloud of pollen from the already dehiscent anthers. During this process, the bees get a pollen shower all over their body, especially on their dorsal side. Further, the bees fly between individual trees in quest of more forage; this foraging activity is important to bring about cross-pollination. The production of a small number of fresh flowers daily at tree level may also compel the pollinator bees to make inter-tree flights and effect cross-pollination. Their body washings indicated that they carry pollen and transfer the same to other flowers they visit. The study suggests that *B. gymnorrhiza* is strictly melittophilous and this mode of pollination is as efficient as ornithophily; but the pollen feeding activity of bees may affect the pollen availability rate for the receptive stigmas at the population level which in turn may affect the natural fruit set rate. However, the bees are reliable pollinators when compared to bird pollinators. The ability of the plant to utilize birds and bees is certainly adaptive and is also essentially required for the survival, colonization and expansion of its geographical range (Tomlinson, 1986).

Tomlinson et al. (1979) stated that small-flowered *Bruguiera* species including *B. cylindrica* is pollinated by butterflies. These authors also mentioned the following characteristics as adaptations for butterfly pollination: small flowers in nearly erect state and displayed to the outside of the tree crown; thin branchlets which are insufficient for bird perching; greenish-yellow petals; flat calyx cup with a small quantity of nectar; and pollen release by delicate, distal stimulation of petals. In this study, the flowers have been found to have three different orientations while all other characteristics remain the same. A careful examination of these characteristics as Faegri and van der Pijl (1979) does not conform to the characteristics of butterfly-flowers. Tomlinson (1986) reported that this species is pollinated by small insects including butterflies. In this study, *B. cylindrica* has been found to be pollinated by bee and wasp species only. *Nomia* bees, *Odynerus* and *Polistes* wasps during nectar collection and the first species also during pollen collection trip all petals of a flower mostly in a single visit; then these insects get a pollen drizzle all over their body, especially on their dorsal side. Further, they fly between individual trees to collect more forage and in so doing they carry pollen on their bodies; this foraging activity is important to bring about self- and cross-pollination. Therefore, *B. cylindrica* is bee- and wasp-pollinated but not butterfly-pollinated. Tomlinson (1986) mentioned that bees and wasps represent a group of pollinators that nest in mangroves, and some populations are completely dependent on mangal for their existence. Ghosh et al. (2008) reported that some wasps and flies are highly dependent on mangroves for nesting. In the light of these reports, it is not unreasonable to suggest that bees and wasps are reliable pollinators since they nest in mangroves and collect forage from the same plants for their nutrition.

In the present study, both the species of *Bruguiera* with specialized floral mechanism offer pollen and nectar as rewards to their pollinators. Pollen grains are very dry even at the time of flower-opening. Their surface sculpture is finely reticulate; they are tricolporate with numerous small and shallow depressions, and easily adhere to the body of insect pollinator so that the latter can easily transfer pollen (Kondo et al., 1987). They contain some protein content and are important in the nutrition of bee pollinators. In *B. gymnorrhiza*, nectar production is continuous in some flowers, while it is not so in some other flowers. Such nectar production pattern indicates that nectar secretion ceases in pollinated flowers, while its secretion is continuous until the stigma loses receptivity or until the flower is pollinated. The production of copious amount of nectar with dilute sugar concentration is the characteristic of bird-pollinated flowers (Baker and Baker, 1983). But, the nectar of *B. gymnorrhiza* is copious with moderate sugar concentration; it is suitable for bee pollinators as the latter tend to prefer sugar concentrations of 30 to 50% (Waller, 1972). The sugars present in the nectar include only hexoses. Roubik (1995) stated that nectar with only hexose sugars is rarely reported. Baker and Baker (1983) segregated bee flowers into those adapted to “short-tongued” bees (with less than six mm in length) and those adapted to “long-tongued” bees. These authors also reported that nectars of flowers pollinated by short-tongued bees are usually hexose-rich, while those pollinated by long-tongued bees are usually sucrose-rich. In line with this, the bees observed have tongues less than five mm length, are short-tongued and utilize the hexose nectar of *B. gymnorrhiza*. De Groot (1953) reported that insects in general and bees in particular require ten essential amino acids - threonine, valine, methionine, leucine, iso-leucine, phenylalanine, lysine, histidine, arginine and tryptophan. The nectar of *B. gymnorrhiza* has three of these essential amino acids, lysine, histidine and arginine. The presence of these amino acids and also other non-essential amino acids, alanine, aspartic acid, glutamic acid, glycine and serine may have a role in giving the “taste” to the nectar (Baker and Baker, 1982). The nectar also has some protein content. Therefore, the nectar of *B. gymnorrhiza* with these amino acids and protein content has high nutritional value and bee pollinators are attracted to this floral source, especially during concentrated flowering period.

In *B. cylindrica*, the nectar is produced in small quantity, hexose-dominant and the sugar concentration is low. Bees and wasps utilize this nectar until exhausted. This observation is in partial agreement with the generalizations made by Baker and Baker (1982; 1983) who stated that the production of a small quantity of nectar with high sugar concentration is the characteristic of bee-flowers, and that the nectars of flowers adapted for pollination by short-tongued bees are hexose-rich, while those adapted for pollination by wasps are sucrose-rich.

Tomlinson (1986) mentioned that pollination may favour outcrossing in *B. gymnorrhiza*. Kondo et al. (1987) reported that this species produces fruit through allogamy, autogamy and geitonogamy and hence, it might be outcrossing and inbreeding. Ge et al. (2003) stated that this species has mixed mating system with outcrossing as a main system. In this study also, *B. gymnorrhiza* has been found to set fruit through allogamy, geitonogamy and autogamy, but all modes are functional only when petal explosion is manipulated; this suggests that pollination is essentially vector-dependent and fruit set is completely a consequence of the foraging activity of bee pollinators. The fallen flowers with some or a few unexploded petals evidenced in this study indicate that wind is not an agent of pollination. Fruit set through vector-mediated autogamy or geitonogamy indicates that *B. gymnorrhiza* is self-compatible and self-pollinating. But, protandrous condition and stigma showing receptivity commencing from day two and extending its receptivity until day four indicate that it is primarily adapted for cross-pollination. The small papilla and mucilage secreted by stigmatic lobes are special adaptations to retain pollen readily; these are especially important for capturing cross-pollen (Kondo et al., 1987). Therefore, *B. gymnorrhiza* with mixed mating system is adapted for pollination by biotic agents, the classes of which may change from time to time in the same habitat depending on the local land use changes. Similar vector-dependent mixed mating system and stigma function exists in *B. cylindrica*. Such a mating system facilitates fruit set in the presence of pollinators even in isolated trees of both the species of *Bruguiera*. With the ability to set fruit through self-pollination, these species can colonize new areas and expand their distribution range. *B. cylindrica* has scattered distribution in the study sites and also here and there it has established small patches representing pure stands. This finding is in agreement with Tomlinson (1986) who stated that it may form pure stands.

In mangrove Rhizophoraceae, the flowers have been reported to contain six or four ovules (Tomlinson, 1986). The flowers are six-ovuled in *B. gymnorrhiza* and four-ovuled in *B. cylindrica*; but only one ovule develops into mature seed in fertilized and fruited flowers in both the species. The production of one-seeded fruits may be due to maternal resource constraint or maternal regulation of seed set. Fruits mature within a month. The persistent and expanded calyx gives protection to the fruit; the mature fruit is well seated within the calyx and hence is not directly exposed to sunlight. Therefore, the calyx has an important role in protecting the fruit from desiccation.

In both the species of *Bruguiera*, the single seed formed in the fruit is not dormant and germinates immediately to produce a cylindrical hypocotyl or seedling, while still on the maternal parent. The hypocotyl emerges out of seed and fruit pericarp and remains naked until it is detached from the parent plant. This is a characteristic of “true viviparous” species (Tomlinson, 1986). While still attached to the maternal parent, the seedling develops chlorophyll and actively photosynthesizes; the parent tree supplies the water and necessary nutrients (Selvam and Karunagaran, 2004). The seedling hangs downward and detaches from the residual fruit, leaving behind its cotyledons, and falls from the maternal parent. Viviparous reproduction allows seedlings to develop some salinity tolerance before being released from the parent tree. This reproduction provides a store of nutrients before the seedlings fall off from the maternal parent and may help in quick rooting in the muddy environment. The seedling characteristics also help to develop buoyancy for dispersal and structural stability for protection against damage (Kathiresan, 2003). Therefore, vivipary is an adaptive feature to overcome the harsh tidal environment for seedling establishment.

The study shows that seedlings of *Bruguiera* fall off the maternal parent freely and plant themselves into the mud or stranded and planted away from parent tree. La Rue and Muzik (1954), Rabinowitz (1978) and Van Speybroeck (1992) reported similarly for this species. Kairo et al. (2001) reported that the mode of seedling dispersal depends on the forest conditions, tides, as well as the stability of the soils. Further, Van Speybroeck (1992) reported that the seedlings can plant themselves into the mud if they are dropped from maternal parent at low water or low tide. He termed it as self-planting strategy. He also reported that the seedlings float to another site to settle and develop if they fall in the water at high tide and he termed it as stranding strategy. The study shows that the seedlings of *Bruguiera* disperse through self-planting and stranding strategies; the former strategy is functional at low tide while the latter strategy is functional at high tide. Van Speybroeck (1992) reported that the self-planting strategy dominates in undisturbed mangrove forest whereas the stranding strategy is dominant in an exploited and open forest. The stranding strategy is especially important for regeneration and colonization of naked or semi-naked habitats and also for expanding the distribution range of the species.

The study found that the Roseringed Parakeet feeds on the softest part of the seedlings of *B. gymnorrhiza* prior to their detachment from the parent tree. The seedlings attacked by this parakeet have not established new plants; their percentage however did not exceed 5%. The feeding on these seedlings by the parakeet may be partly attributable to the scarcity of food. There is no seedling predation prior to detachment from the maternal parent in *B. cylindrica.* Sousa and Mitchell (1999) reported that after detachment from the maternal parent, seedlings experience mortality due to crab predation. Crabs prefer to feed on small propagules as the latter facilitate easy burial in burrows, have high nutritive value and low concentration of inhibiting chemicals such as tannins. Since the seedlings of both the species of *Bruguiera* are long, crabs may not utilize them as food source but further study is required to confirm this.

Avicennia alba, Avicennia marina and ***Avicennia officinalis***. All the three *Avicennia* species studied are principally polyhaline evergreen tree species. *A. alba* and *A. marina* are small trees while *A. officinalis* is a tall tree. These tree species show flowering response to monsoon showers in June; the first monsoon showers seem to provide the necessary stimulus for flowering. Opler et al. (1976) and Ewusie (1980) have reported such a flowering response to light rains in summer season in a number of plants occurring in coastal environments. The flowering period extends until August in all the three species of *Avicennia* at the study sites, indicating that the flowering season is only for three months in a year. On the contrary, Wium-Andersen and Christensen (1978) reported that in *A. marina*, flowering occurs during April-May. Further, Mulik and Bhosale (1989) noted that the flowering in this species is from April to September. These authors also mentioned that the flowering occurs during March-July in *A. officinalis*. The variation in the schedule and length of flowering season in these species may be a response to local environmental conditions and to avoid competition for the available pollinators depending on the flowering seasons and population size of the constituent plant species which vary with each mangrove forest. In all the three species, the flowers are borne either in terminal or axillary inflorescences. But, the average number of flowers per inflorescence varies with each species; it is the highest in *A. alba*, moderate in *A. marina* and the least in *A. officinalis*. This flower production rate at inflorescence level may serve as an important taxonomic characteristic for the identification of these three species.

In all, the flowers are strongly protandrous and the stamens with dehisced anthers over-arch the stigma. The stigma shows post-anthesis growth. It is erect and seated in the centre of the flower in *A. alba* and *A. marina* while it is bent and situated below the adaxial corolla lobe in *A. officinalis*. The erect stigma does not change its orientation throughout the flower life in *A. alba* and *A. marina* while the bent stigma becomes erect on day three. The stigma is bifid and appressed on the day of anthesis in all the three species; it remains in the same state also on day two in *A. officinalis*. The stigma commences receptivity by diverging in dorsi-ventral plane; it is receptive on day two and three in *A. alba* and *A. marina*, and on day three, four and five in *A. officinalis*. The timing of commencement of stigma receptivity in *A. officinalis* strongly contradicts with an earlier report by Subba Reddi et al. (1995) that the stigma attains receptivity three hours after anthesis with the bent stigma becoming erect. In *A. officinalis*, stigma behaviour is more advanced towards achieving cross-pollination. In all the three species, self-pollination of individual flowers is unlikely on the day of anthesis due to protandry but the stamens with dehisced anthers over-arching the stigma may facilitate the fall of pollen on the receptive stigma when the latter attains receptivity. In effect, self-pollination may occur and the same is evidenced through fruit set in bagged flowers without manual self-pollination. Further, the sequence and synchrony of flowering, and pollinator behaviour at tree level contribute to geitonogamy (Clarke and Meyerscough, 1991). Hand-pollination results indicate that it is self-compatible and fruit set occurs through autogamy, geitonogamy and allogamy. The hermaphroditic flowers with strong protandry and long period of flower life in these species suggest that they are primarily adapted for cross-pollination. Clarke and Meyerscough (1991) also reported that *A. marina* is protandrous, self-compatible and self-pollinating but the fruits resulting from spontaneous self-pollination showed a higher rate of maternal abortion reflecting an inbreeding depression. Coupland et al. (2006) reported that in *A. marina*, autogamy is most unlikely and emphasized the importance of pollen vectors to the reproductive success. This report is not in agreement with the results obtained in hand-pollination experiments on *A. marina*. Primack et al. (1981) suggest that protandry promotes out-crossing in mangroves, and that insect pollination facilitates it. They also suggested that geitonogamy in coastal colonizing plants would allow some fruit set in isolated colonizing plants, and thereafter the proportion of such pollinations would decline as pollen is transferred between plants. Pollen transfer between plants in such situations would still result in sibling mating. However, this is counteracted by dispersal of propagules, canopy suppression of seedlings and irregular yearly flowering among trees in close proximity. Clarke and Meyerscough (1991) reported that in *A. marina*, some trees flower and fruit every year while some others do not flower every year. Those with complete canopy crops did not produce another large crop the following year. A similar pattern observed within a tree where fruit is produced on one branch and in the following year heavy flowering shifts to another branch. In the present study, all the three species of *Avicennia* flowered annually and the flowering is uniform on all branches within a tree. The study suggests that annual mass flowering, protandry, self-compatibility and self-pollination ability are important adaptations for *Avicennia* species to successfully colonize new areas and expand their distribution range as pioneer mangroves.

All the three species of *Avicennia* are hermaphroditic and have similar floral architecture. In *A. officinalis*, the flowers are foetid and slightly zygomorphic while in the other two species, they are scented and actinomorphic. In all, the flowers are of open type and shallow with small aliquots of nectar which is exposed to rapid evaporation resulting in increased nectar sugar concentration. Corbet (1978) considered these characteristics as adaptations for fly pollination. Hexose-rich nectar is present in *A. alba* and *A. marina* while sucrose-rich nectar in *A. officinalis*. Hexose-rich nectar is the characteristic of fly- and short-tongued bee-flowers while sucrose-rich nectar is the characteristic of wasps and butterflies (Baker and Baker, 1982, 1983). The nectar sugar concentration is high and ranged from 38 to 40% in all the three *Avicennia* species. Cruden et al. (1983) reported that high nectar sugar concentration is the characteristic of bee-flowers while low nectar sugar concentration is the characteristic of butterfly-flowers. Baker and Baker (1982) reported that the floral nectar is an important source of amino acids for insects. Dadd (1973) stated that insects require ten essential amino acids of which arginine, lysine, threonine and histidine are present in the nectar of *A. officinalis*. He also reported that proline and glycine are essential amino acids for some insects; these two amino acids are also present in the nectar of *A. officinalis*. He further stated that other amino acids such as alanine, aspartic acid, glutamic acid, glycine and serine while not essential do increase insect growth. All these amino acids are also present in the nectar of *A. officinalis*. Shiraishi and Kuwabara (1970) reported that proline stimulates salt receptor cells in flies. Goldrich (1973) reported that histidine elicits a feeding response while glycine and serine invoke an extension of the proboscis. The nectars of *A. alba* and *A. marina* have not been analyzed for amino acids and hence this aspect has not been discussed.

The flowers of all the three species of *Avicennia* with differences in their structural and functional characteristics as stated above have been able to attract different classes of insects - bees, wasps, flies and butterflies. Of these, bees while collecting pollen and nectar while all others collecting nectar effected pollination and their ability to carry pollen has been evidenced in their body washings. Flies are known as short distance fliers and such behaviour largely results in autogamy or geitonogamy. Since these flies visit the flowers as large groups, there is automatically a competition for the available nectar which is secreted in small aliquots on the petals of all the three *Avicennia* species. In consequence, they shift from tree to tree in search of nectar forage and in the process they contribute to both self- and cross-pollination. All other insects are habitual long-distance fliers and affect both self- and cross-pollination. An earlier report by Subba Reddi et al. (1995) showed that only bees and flies are the pollinators of *A. officinalis* at the study sites. Tomlinson (1986) mentioned that *Avicennia* flowers are bee-pollinated. In Australia, *A. marina* is pollinated by ants, wasps, bugs, flies, bee-flies, cantharid beetles and moths (Clarke and Meyerscough, 1991). It is surprising to note that thrips are absent both in bud and flower stage in all the three species of *Avicennia*. A study on this aspect is needed to understand why thrips avoided these flowers for breeding or for forage collection.

Tomlinson (1986) documented that A. *alba, A. marina* and *A. officinalis* have very similar flowers and hence may well be served by the same class, if not by the same species of pollinators; when these species grow together, there is evidence of non-synchrony in flowering times, which might minimize the competition for pollinators (probably bees) and at the same time spread the availability of nectar over a more extended period. In the present study, these plant species grow together, flower synchronously, but served by the same classes of insects. There is no competition for pollen among different classes of insects since only bees collect pollen while all other classes of insects collect only nectar. Fly pollinators with their swarming behaviour at the flowers may enable the plant species to set fruit to the extent possible. Flies and bees are usually consistent and reliable when compared to wasps and butterflies. Therefore, the study shows flies and bees play an important role in the success of sexual reproduction in all the three species of *Avicennia.* Despite being pollinated by different classes of insect pollinators and having the ability to self-pollinate even in the absence of insect activity as evidenced in bagged flowers, the natural fruit set stands at 42-58% in these plant species. This low fruit set could be due to maternal abortion of self-pollinated fruits as reported by Clarke and Meyerscough (1991), non-availability of sufficient pollen to receptive stigmas due to pollen feeding activity of bees and the nutritional resource constraint to the maternal parent. Coupland et al. (2006), while reporting on fruit set aspects of *A. marina* in Australia mentioned that fruit set is not pollinator limited but resource limited.

In Avicenniaceae, the flowers have been reported to contain four ovules (Tomlinson, 1986). In the present study, all the three species of *Avicennia* are four-ovuled but only one ovule develops into mature seed in fertilized and fruited flowers as in Rhizophoraceae. The production of one-seeded fruits may be due to maternal resource constraint or maternal regulation of seed set. Fruits grow and mature within five-six weeks in *A. alba* and within four weeks in the other two *Avicennia* species. The duration of fruit maturation is not in agreement with the report of Wium-Andersen and Christensen (1978) who stated that the development from flower bud to mature fruit takes a few months. The calyx is persistent in all the three species but it does not expand to enclose the growing fruit. Therefore, the calyx has no role in sheltering or protecting the fruit. As the fruit is a leathery capsule, it does not require any protection from the calyx.

The single seed formed in the fruit is not dormant and germinates immediately to produce chlorophyllous seedling, which remains within the fruit, while still on the maternal parent. This is a characteristic of “crypto-viviparous” species; a similar situation exists in other genera such as *Aegiceras, Aegialitis, Nypa* and *Pelliciera* (Tomlinson, 1986). In all these species, fruit is the propagule; the seedling occupies the fruit cavity. The chlorophyllous seedling actively photosynthesizes while the maternal parent supplies the water and necessary nutrients (Selvam and Karunagaran, 2004). In *Avicennia* species, the propagules are small, light and the entire embryo is buoyant after detachment from the maternal parent. Gradually, the fruit pericarp is lost exposing the leathery succulent cotyledons to tidal water. Rabinowitz (1978) reported that *A. marina* has an absolute requirement for a stranding period in order to establish since its propagules always float in tidal water. He also felt that the propagules must have freedom from tidal disturbance in order to take hold in the soil. In consequence, this species is restricted to the higher ground portions of the swamp where the tidal inundation is less frequent. In the present study, *Avicennia* species exhibit self-planting strategy at low tide and stranding strategy at high tide. However, their seedlings disperse widely in tidal water but establishment is mainly stationed in the polyhaline zone. Duke et al. (1998) reported that *Avicennia* seedlings disperse widely and are genetically uniform throughout their range. In the study areas, genetic studies are required to know whether all the three species studied are genetically uniform. When the seedlings settle, radicle penetrates the sediment before the cotyledons unfold. The first formal leaves appear one month after germination and the second pair one to two months (Wium-Andersen and Christensen, 1978).

Coupland et al. (2006) reported that *Avicennia* propagules are a rich source of nutrients and attract a diverse range of insect predators which in turn influence the rate of seedling maturation. Resource constraints and insect predation on developing fruit and seedling may both act to reduce fruit set. In *A. marina* and *A. germinans*, the seedlings tend to be high in nutritive value and have relatively few chemical defences (Smith, 1987; McKee, 1995). These species tend to exhibit a pattern of very rapid initial predation (Allen et al., 2003). In the present study, seedling predation has been evidenced in *A. alba* and *A. marina* only; in both the species, the Rose-ringed Parakeet, *Psittacula krameri* attacks propagules prior to their detachment from the maternal parent. Seedling predation by crabs after detachment from the maternal parent may be expected since different species of crabs have been found in the study areas. Therefore, seedling predation may reduce the success of seedling establishment in all the three species of *Avicennia*.

Aegiceras corniculatum. The plant is a mesohaline evergreen species. The flowering occurs during the dry season; sporadic flowering also occurs at other times of the year but it is especially significant during the rainy season. The floral characteristics such as morning anthesis, scent production, zygomorphic symmetry, short-tubed corolla with sexual organs exposed, pollen structural features and nectar production are adaptations for pollination by any class of animals (Baker and Baker, 1983; Cruden et al., 1983). Different workers reported different insects as pollinators of *A. corniculatum* - bees without mentioning the species by Tomlinson (1986); the bees *Trigona iridipennis* and *Pseudapis oxybeloides* at Coringa mangrove forest by Solomon Raju (1989); butterflies, bees, wasps and flies, and also birds in the Orissa mangrove forest by Pandit and Choudhury (2001). The present study shows that bees, wasps, flies and butterflies are the pollinators; *Xylocopa* bees are the most efficient pollinators due to their ability to collect forage quickly from each umbel and to their quick mobility between plants for want of more forage. The bees are pollen and nectar collectors while all others are nectar feeders. Pollen is a source of protein for bees and the pollen of *A.*

corniculatum with some protein content is important for them. The small volume of nectar with high sugar concentration consisting of only two hexose sugars, fructose and glucose in *A. corniculatum* is stated to be a requirement for bees, flies and butterflies while sucrose-rich nectar is the requirement for wasps (Baker and Baker, 1983). This study shows that wasps also utilize short-tubed flowers with nectar containing only hexose sugars. Further, the nectar contains four of the ten essential amino acids required by insects (De Groot, 1953). They are arginine, lysine, threonine and histidine. It also has some non-essential amino acids. These essential and non-essential amino acids in the nectar of *A. corniculatum* serve as important nutrient source for all the insects. Near synchronous anthesis in one umbel and acropetal succession of flowers resulting in the extension of flowering period are energetically beneficial and provide forage continuously until the cessation of flowering. All the insects carry pollen and pollinate the flowers without fail. *A. corniculatum* with its unspecialized flowers is capable of utilizing the locally available insect species for fruit set; the insects are especially important for cross-pollination. The pollen grains being light and dry, and small in size facilitated by the medifixed versatile anthers are carried away by wind action and this wind-driven pollen movement also contributes to a small percent of fruit set. Therefore, both insects and wind are the pollinators of this plant.

In *A. corniculatum*, the anthers and stigma of a flower mature simultaneously; anthers dehisce an hour after anthesis while stigma remains receptive for the next two days. Pandit and Choudhury (2001) mentioned that the flowers of this plant would be able to self-pollinate with the simultaneous anther dehiscence and stigma receptivity, and with the stigma position at the level of the anthers. The plant is self-compatible and capable of autogamy, but suggested that insect pollinators are required for a higher level of fruit set. They also substantiated their suggestion by stating that pollen-ovule ratio falls within the range of facultative xenogamy according to Cruden (1977). Further, these authors observed low levels of genetic variation in this plant occurring in China; this may be an indication that fruit set in this plant is largely a function of self-pollination. Solomon Raju (1989) also reported that autogamy is a mode of self-pollination for fruit set in this plant. In the present study, hand-pollination results indicate that fruit set occurs through autogamy, wind and insects, but fruit set is highest only in the flowers pollinated by insects. All of this indicates that the plant though self-compatible and capable of setting fruit through autogamy is largely dependent on insects for maximizing fruit set rate. The pollen viability and stigma receptivity for more than three days indicate are additional adaptations to achieve cross-pollination through foraging activity of insects. In this context, Primack and Tomlinson (1980) argued that if the plant is primarily a colonizing species, it would retain the need for self-fertility if it is to establish populations in isolated localities. Therefore, *A. corniculatum* with self-compatible option would be able to establish populations even in isolated localities and utilize locally available flower visiting insects for both self- and cross-pollination.

In *A. corniculatum*, flower bud abortion is negligible and its occurrence may be due to defective origin. Numerous flattened ovules are embedded in the rounded, somewhat fleshy, and short-stalked free central placenta; it may cause an underestimation of ovule number. The present study shows that the ovules in an ovary are only thirty five but only one ovule produces seed in each fruit. Fruit maturation takes a month or slightly more than a month’s time. Seed is not dormant and produces a hypocotyl within the fruit pericarp. The fruits hang downwards in this plant whereas the fruits stand upwards in *Aegialitis rotundifolia*. The entire fruit of *A. corniculatum* falls off when due for dispersal. Hypocotyls float only if they are with the fruit pericarp. Self-planting and stranding strategies are effective for the dispersal and establishment of hypocotyls. Therefore, the plant displays scattered occurrence and also forms pure stands in certain mesohaline areas of the mangrove forest. Bosire et al. (2005) reported higher rate of crab predation in this plant. In the present study, this aspect was not examined but the hypocotyls being small in size may contain high nutrient content and low fiber content may attract crab predators prior to their establishment.

Aegialitis rotundifolia. The genus *Aegialitis* represents only two shrub species, *A. annulata* and *A. rotundifolia*. It is recently segregated as the family Aegialitidaceae because of some distinctive features from other genera of Plumbaginaceae. Some features include anomalous secondary thickening, abundant sclereids, incipiently viviparous seeds, monomorphic pollen and homostylous flowers (Weber-El Ghobary, 1984; Tomlinson, 1986). The two species do not occur together in the same forest and have distribution in different parts of the world. *A. annulata* is distributed in Australia and eastern Malaysia (Tomlinson, 1986) while *A. rotundifolia* in South Africa and South-East Asia (Kathiresan and Bingham, 2001). *A. rotundifolia* has been reported to occur in Burma, Bengal and the Andamans by Tomlinson (1986). Later, Naskar and Mandal (1999) reported this species as occurring in the Sundarbans, Andaman and Nicobar islands and Mahanadi Delta of Orissa only. Ramasubramanian et al. (2003) have not mentioned about the occurrence of *A. rotundifolia* in their published book on the mangrove flora of Krishna and Godavari deltas of Andhra Pradesh. The present study revealed the presence of *A. rotundifolia* in Nachugunta Reserve Forest of Krishna Mangroves in Andhra Pradesh and hence it is the first record of *A. rotundifolia* from Andhra Pradesh.

Tomlinson (1986) stated the fact that *Aegialitis* species prefer or require exposed sites and withstand waves and tidal action. Further, he also mentioned the fact that *A. rotundifolia* does not occur within closed mangrove communities but it may occur as back mangal if soil is highly saline. Aksornkoae et al. (1992) also reported similarly about the habitat requirements of *A. rotundifolia*. At the study site also, this species occurs in seaward, euhaline and exposed sites. As the habitat of *A. rotundifolia* is highly saline, salt in high concentrations in plant tissues is toxic and hence, must be excluded by some mechanism. The absorbed salt is excreted metabolically via salt glands present on the leaf blade (Scholander, 1968). The salt evaporates or crystallizes in a conspicuous manner on the surface of leathery leaf blade of *A. rotundifolia*. Later, the crystallized salt blows away or washes off during cool periods by the absorption of atmospheric moisture and by rain. Therefore, *A. rotundifolia* with salt excretion mechanism is highly specialized to withstand high saline soils.

The plant is a dry season bloomer but it completes flowering prior to the onset of extreme dry conditions in the month of May. During this period, fluvial discharge from rivers to the sea is almost negligible and this would result in increased salinity of seawater. A steep increase in salinity levels can be expected at the site of the plant which is characteristically seaward in occurrence. Increased salinity of seawater reportedly prevents fruiting and causes senescence of immature flowers and buds (Qureshi, 1993). This may be an important factor for the plant to cease flowering by the mid-April and supply the available resources to the growing fruits to realize maximum fruit set.

A. rotundifolia species with anthesis during morning hours and odourless flowers indicates that it is adapted for pollination during daytime. The floral characteristics of this plant species such as short-tubed corolla with anthers at the flower entrance, the styles and stigmas situated slightly below the anthers, production of slightly moderate volume of nectar with high sugar concentration and the presence of only hexose sugars in nectar are adaptations for bee-pollination (Baker and Baker, 1983; Opler, 1983). Further, the nectar has the essential amino acids such as arginine, lysine, phenylalanine, threonine, tryptophan, valine and histidine; and also some non-essential amino acids. De Groot (1953) showed that insects in general and honeybees in particular require ten essential amino acids and seven of them are present in the nectar of this plant. The pollen also has some protein content. The flower visitors recorded are exclusively honeybees and stingless bees; the latter is also honey producers. These bees collect both pollen and nectar from the flowers. They carry pollen on their bodies and pollinate the flowers while probing for the forage. As they require more quantity of forage for honey production and brood rearing, they collect forage from as many flowers as available on *A. rotundifolia* and hence, contribute to both self- and cross-pollination. Naskar and Mandal (1999) mentioned that this plant is pollinated by the honey bee, *Apis dorsata* in the Sundarban mangroves. Bhattacharya et al. (2006) also noted that the pollen of this plant is dominant in honey collected from the Sundarbans region. Therefore, *A. rotundifolia* species is primarily melittophilous.

A. rotundifolia species flowers are weakly protandrous, self-compatible and self-pollinating. The protandry does not contribute to autogamy in the first two hours period of flower life as the stigmas lack receptivity during that period. Gradually, the stigmas diverge and stand away from the anthers while they attain receptivity to pollen. Individual flowers with this situation produce fruit through autogamy with the aid of wind or honeybees. The pollen grains are very large and fall down on the stigmas gravitationally due to wind action, the result of which is autogamy; if there are flowers of the same age side by side in the same or adjacent inflorescences, geitonogamy may occur. However, the flower function with reference to protandry, movement of stigmas and duration of stigma receptivity suggests that the plant is primarily evolved for outcrossing. Hand-pollination results also indicate the same and the fruit set rate is highest in open-pollinations which are largely a function of foraging activity of bees. Despite high fruit set rate in this plant, its population size is small. Pollination among the individuals of this small population may lead to a reduction in genetic diversity and molecular studies on its genetic structure would enable to know the existing level of genetic variation. Transplanting the propagules from other mangrove forests like mangrove of the Mahanadi Delta of Orissa and the Sundarbans to the study site would help to enhance gene flow in order to enable the plant to build up a stable and sustainable population size.

The flowers produce singled-ovuled ovary and the ovule invariably produces a single seed in fertilized flowers. This character is advantageous for the plant to save and use the resources for higher fruit set rate. Seed is not dormant and produces hypocotyl within the fruit pericarp while still on the parent plant; it is a characteristic of crypto-viviparous species (Carey, 1934; Das and Ghose, 2003). The fruit with hypocotyl inside grows upward like the upwardly growing naked hypocotyl of *C. decandra*. Since the hypocotyl is concealed, the entire capsule-like fruit falls off when due for dispersal. Fruit pericarp is essential for the hypocotyl to float until it is settled. Self-planting and stranding strategies are functional for the dispersal of hypocotyls. But, field studies indicate that only a few of those hypocotyls which have fallen at the parental sites settled well and showed further growth. Clarke and Kerrigan (2002) reported that the small-sized hypocotyls contain high nutrient content and low fiber content. The crabs prefer to consume such hypocotyls and feed on them prior to establishment. They also reported that 80% of the propagules failed to establish due to predation by crabs in *Aegialitis annulata*. In the study site of *A. rotundifolia* also, crabs are common and they may be consuming most of the hypocotyls prior to their establishment and hence, affecting the recruitment process of the plant.

CONCLUSIONS

Both viviparous and crypto-viviparous species exhibit mixed mating system and adaptations for entomophily. In case of *B. gymnorrhiza*, the floral features suggest ornithophily but locally the plant is found to be melittophilous. The mixed mating system coupled with entomophily appears to be adaptive for the success of sexual reproduction in harsh environment which is characteristic of mangrove forest. The seed is usually the stage of the life cycle at which dispersal and the colonization of new areas occurs. It contains a reserve of food, providing the embryo with a temporary continuation of maternal support. In contrast, in both true and crypto-viviparous tree species, the seed lacks dormancy and does not perform any of these roles. The unit of dispersal in these species is not the seed but the young seedling (hypocotyl); it is naked in true viviparous species while it remained within the fruit pericarp in crypto-viviparous species suggesting that the zygote is not dependent on stored nutritional support from the endosperm or carpal tissues, but instead may be nourished directly from the maternal plant. In all the plant species, the dispersal of propagules takes place via self-planting and stranding strategies. The self-planting strategy is important in undisturbed sites while the stranding strategy is effective in exploited and open forest sites of mangroves. Therefore, the detailed information included in this paper is useful for framing effective measures for conservation and management of the studied mangrove plants as these are the characteristic species of mangrove forest. The study further provide basis for taking up extensive studies on mangrove plants for the sustainability of mangrove forest.

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DE GRUYTER OPEN

ANALYSING LEARNING AT THE INTERFACE OF SCIENTIFIC AND TRADITIONAL ECOLOGICAL KNOWLEDGE IN A MANGROVE ECOSYSTEM RESTORATION SCENARIO IN THE EASTERN COAST OF TANZANIA

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DOI: 10.2478/trser-2013-0027

KEYWORDS: community-based participatory monitoring, mangrove ecosystem, scientific indicators, context-based learning, scientific knowledge, traditional ecological knowledge, traditional ecological indicators, under-labouring.

ABSTRACT

Records from community-based coastal management initiatives indicate that local communities who are key actors in activities that aim at safeguarding the health status of terrestrial and marine ecosystems face a lot of challenges associated with adapting and applying indicators that are scientifically abstracted and methodologically too reified, given varying social, contextual and technical conditions prevailing amongst them.

This paper brings into view possible challenges of adapting and applying scientific indicators in community-based monitoring of mangrove ecosystem and suggests a new approach that may lead to development of indicators which are less reified, more congruent to users (coastal communities) and likely to attract a wider social learning in the mangrove restoration context. It also sets a bridge for scientific institutions (including universities), to understand various social, cultural and contextual needs that determine epistemological access between them and local communities, which need to be addressed prior to engaging target communities in participatory monitoring programmes.

The paper attempts to analyse learning at the interface of knowledge that scientific institutions produce and the potential knowledge that exists in local context (traditional ecological knowledge) for purposes of widening and improving knowledge sharing and safeguarding the health status of mangrove species and fisheries that use them as key habitats.

The paper stems from a study which employs processes of abstraction and experiential learning techniques such as Experiential Learning Intervention Workshop carried out in 2012, to unlock knowledge that local communities have, as an input for underlabouring existing scientific indicators in the eastern coast of Tanzania.

It brings into view the need to consider contextual realities on ground, the level of education that the participating group has, the minimum level of participation that is required, structures that govern coastal monitoring practices at local level and the need for scientific institutions to consider the knowledge that local people have as an input for enhancing or improving coastal monitoring, especially monitoring of mangrove and fishery resources.

The paper finally comes up with a framework of indicators which is regarded by coastal communities as being less reified, more contextually and culturally congruent and which can easily be used in detecting environmental trends, threats, changes and conditions of mangrove and fisheries resources, and attract wider social learning processes.

ZUSAMMENFASSUNG: Analyse des Lernens an der Schnittstelle wissenschaftlicher und traditioneller ökologischer Kenntnisse in einem Mangroven-Ökosystem Renaturierungs-Szenarium an der Ostküste von Tanzania.

Unterlagen gemeinschaftsgestützter Initiativen von Küstenmanagement zeigen, dass die lokalen Gemeinschaften, die Schlüsselstelle in der beabsichtigten Sicherung des Gesundheitszustandes der terrestrischen und marinen Ökosysteme einnehmen, vor einer Reihe von Herausforderungen stehen, die verbunden sind mit der Anpassung und Umsetzung von Indikatoren, die wissenschaftlich abstrakt und methodisch zu reifiziert sind angesichts der sozialen, kontextabhängigen und technischen Bedingungen, die zwischen ihnen vorherrschen.

Die vorliegende Arbeit veranschaulicht mögliche Herausforderungen zur Anpassung und Anwendung wissenschaftlicher Indikatoren in einem gemeinschaftlich angelegten Monitoring von Mangroven Ökosystemen und regt eine neue Herangehensweise an die Entwicklung von Indikatoren an, die weniger reifiziert sind und mehr den Nutzern (die Küstengemeinden) enstprechen, wodurch sie wahrscheinlich ein weiteres soziales Lernen im Kontext der Renaturierung der Mangroven aktivieren werden. Ebenso legt es eine Brücke zu wissenschaftlichen Institutionen (einschließlich Universitäten), verschiedene soziale, kulturelle und kontextabhängige Notwendigkeiten zu verstehen, die den epistemologischen Zugang zwischen ihnen und den lokalen Gemeinschaften bestimmen, und angesprochen werden müssen, bevor die Zielgemeinden an dem participativen Monitoringprogramm beteiligt werden.

Die Arbeit macht den Versuch, das Lernen an der Schnittstelle der Erkenntnis dessen zu analysieren, was die wissenschaftlichen Institutionen erarbeiten und dem potentiellen Wissen, das im lokalen Kontext existiert (traditionelles ökologisches Wissen), in der Absicht die gemeinsame Nutzung des vorhandenen Wissens zu erweitern und zu verbessern sowie den Gesundheitszustand der Arten und der Fischereiwirtschaft zu sichern, die Magroven als Schlüsselhabitate nutzen.

Die Arbeit rührt von einer Studie her, die sich mit Abstraktionprozessen und Techniken des Lernens aus Erfahrung, wie Experiential Learning Intervention Workshop (ELIW)/Workshop zur Vermittlung von erfahrungsgemäßem Lernen, beschäftigt, um das in den lokalen Gemeinschaften vorhandene Wissen als einen Beitrag zu den unter Arbeitsbedigungen existierenden wissenschaftlichen Indikatoren an der Ostküste von Tanzania zu erschließen.

Es veranschaulicht die Notwendigkeit, die Tatsachen an der Basis im Kontext zu sehen und zwar den Stand der Bildung, den die teilnehmende Gruppe hat, den minimalen Stand der erforderlichen Teilnahme, die Struktur, die Monitoring Praktiken auf lokaler Ebene beherrscht sowie die Notwendigkeit für wissenschaftliche Institutionen die vorhandenen Kenntnisse der lokalen Bevölkerung in Betracht zu ziehen als einen Beitrag zur Leistungsförderung oder zur Verbesserung des Küstenmonitorings, insbesondere das Monitoring der Mangroven und der Fischereiressourcen.

Schließlich bringt die Arbeit ein Bezugssystem von Indikatoren zur Sprache, das bei den Küstengemeinschaften als weniger reifiziert und mehr kontextabhängig sowie kulturell entsprechend ist. Zu dem kann es leicht angewendet werden bei der Erkennung von Umwelttrends, Gefahren, Veränderungen, Zustand der Mangroven und Fischereiressourcen, und findet Beachtung bei weiteren sozialen Lernprozessen.

REZUMAT: Analiza învăţării la limita dintre cunoştinţele ştiinţifice şi cele ecologice tradiţionale, în scenariul restaurării unui ecosistem de mangrove de pe coasta estică a Tanzaniei.

Informaţii/mărturii ale iniţiativelor de management de coastă, bazate pe comunitate indică faptul că personajele-cheie ale activităţilor care au ca scop protejarea stării de sănătate a ecosistemelor terestre şi marine - comunităţile locale, se confruntă cu o mulţime de provocări legate de adaptarea şi aplicarea indicatorilor, care sunt din punct de vedere ştiinţific şi metodologic prea abstracţi, având în vedere condiţiile sociale, contextuale şi tehnice existente.

Această lucrare aduce în atenţie posibile provocări de adaptare şi punere în practică a indicatorilor în monitorizarea ecosistemelor de mangrove, bazată pe comunitate şi sugerează o nouă abordare care ar putea duce la dezvoltarea indicatorilor, care sunt mai puţin abstracţi, mai apropiaţi de utilizatori (comunităţile de coastă) şi de natură, să atragă o învăţare socială mai largă în contextul restaurării mangrovelor. De asemenea, aceasta stabileşte o punte de legătură între instituţiile ştiinţifice (inclusiv universităţi), pentru o mai bună înţelegere a diverselor nevoi sociale, culturale şi contextuale, care determină accesul epistemologic între acestea şi comunităţile locale, care trebuie să fie abordate înainte de a introduce comunităţile ţintă în participarea în cadrul programelor de monitorizare.

Lucrarea reprezintă o încercare de analiză a învăţării la interfaţa cunoştinţelor pe care instituţiile ştiinţifice le produc şi potenţialul de cunoaştere, care există în contextul local (cunoştinţe ecologice tradiţionale) în scopul lărgirii şi îmbunătăţirii schimbului de cunoştinţe şi protejarea stării de sănătate a speciilor de mangrove şi a pescăriilor care le folosesc ca habitate cheie.

Lucrarea provine dintr-un studiu care utilizează procese de abstractizare şi tehnici de învăţare din experienţă, cum ar fi Experiential Learning Intervention Workshop (ELIW) pentru a debloca cunoştinţele pe care comunităţile locale le au, ca un impuls pentru indicatorii ştiinţifici mai puţin eficienţi existenţi pe coasta de est a Tanzaniei.

Studiul aduce în prim plan necesitatea de a lua în considerare realitatea din teren, nivelul de educaţie al grupului participant, nivelul minim de participare care este necesar, structura care guvernează practicile de monitorizare de coastă la nivel local şi necesitatea ca instituţiile ştiinţifice să ia în considerare cunoştinţele pe care localnicii le au ca şi un plus pentru consolidarea sau îmbunătăţirea monitorizării de coastă, mai ales a monitorizării mangrovelor şi a resurselor piscicole.

Lucrarea vine, în cele din urmă, cu un model de indicatori care este considerat de către comunităţile costiere ca fiind mai puţin abstract, mai contextual şi congruent cultural, indicatori care pot fi folosiţi cu uşurinţă în detectarea tendinţelor de mediu, a ameninţărilor, schimbărilor şi care condiţionează mangrovele şi resursele piscicole şi atrag procese sociale mai ample de învăţare.

INTRODUCTION

Learning occurs through conscious and unconscious processes (Breen, 2013) and is not detached from day to day human practices (Lotz-Sisitka, 2012). Much lies in the kind of knowledge that is adapted or generated in various settings as a solution for emerging social, economic, and environmental challenges in the social world.

Debates regarding how people learn can be dated back to the era of Greek philosophers (Socrates-469-399 B.C., Plato 427-347 B.C., and Aristotle 384-322 B.C.) and have since then been filtered to reflect not only philosophical, psychological and biological views but also different dimensions and contexts such as social, cultural, economic, and ecological (Hammond et al., 2001; Reed et al., 2006; Mostert et al., 2007; Pahl-Wostl et al., 2007; Muro and Jeffrey, 2008).

The past two decades have witnessed an increase in global, regional, and local efforts emphasizing the need to create and raise awareness among users of natural resources (particularly mangroves, fisheries, seas grasses, and coral-reefs in different parts of the world). This awareness is especially necessary for those at the grassroots level in low income countries because they may become involved in the periodic detection of environmental trends, threats, conditions, and changes of coastal and marine resources (commonly known as periodic-monitoring); and thus exposing them to "learning by doing"; an approach which encourages learning by taking part in the practice (NEECS, 2005-2009).

Some of the key reasons that underpin the justification for this form of community-based learning are that coastal areas contain diverse and productive habitats necessary for sustenance and subsistence of the world's poor who live around them. It is also recognised that coastal resources are vital for many local communities and indigenous people (UNCED, 1992; NICEMS, 2003). Such participatory monitoring practices are thus carried out to safeguard the health status of the coastal environment, due to increasing pressures from the demand for coastal resources by surrounding local communities (Wagner, 2005; NICEMS, 2003) and the need to support involved communities in order to understand both the value of ecological systems that surround them and assume a leading role in protecting them.

Lotz-Sisitka (2012) argues that learning which takes place in community-based natural resource management contexts encourages knowledge sharing, experimentation, reflective practice, problem solving, effective monitoring and informed planning, leading to behavioural change and trust. Leys and Vanclay (2010) view such forms of learning as an approach that can strengthen communities' capacity to collectively manage ecosystems sustainably.

While learning through direct involvement in practice is necessary (Kuper et al., 2009), much lies on the methods and the indicators employed by development experts and scientists in participatory natural resource management initiatives such as monitoring of mangroves resources and fisheries. In East Africa, (particularly Tanzania where Integrated Coastal Management Programmes were initiated in early, mid and late 1990s under internal and external funding) specific monitoring plans were developed by scientific institutions to guide community-based monitoring practices (KICAMP, 2005). The said monitoring plans contained scientific indicators and methodologies adapted from the Survey Manual for Tropical Marine Resources (English et al., 1994) and were believed to be "in line with those put forward by the Science and Technical Working Group (STWG) of Tanzania Coastal Management Partnership" (KICAMP, 2005).

After several years of implementing community-based monitoring plans, it was observed that local participants (coastal communities) struggled to "understand and apply" the scientific framework of indicators (KICAMP, 2004). This observation was affirmed in 2005, when one of the studies carried out along the eastern Coast of Tanzania indicated that there was no effective monitoring of coastal and marine resources in the area. It thus implies that "learning by doing" did not successfully play out as previously envisaged.

Campbell (2000) affirms that problems are likely to emerge when facilitating firms and organisations prioritize natural scientific norms and approaches in community-based initiatives. Proceedings from the scientific forum on Integrated Coastal Management issues in Tanzania indicate that scientific knowledge was being "presented in a manner that was too complicated" and a form that tended to limit understanding and access to information for management purposes (TCMP, 1998).

However, this paper does not suggest that the adaption and application of scientific indicators and methodologies in the local context constrain the learning process. It rather raises a concern whether or not conditions that necessitate creation or adaption of scientific knowledge are properly addressed by development experts and scientists prior to involving or engaging target communities in the participatory monitoring of coastal and marine resources, particularly the mangroves and fisheries that use them as key habitats. This paper seeks to communicate to different players who are involved in coastal-based monitoring practices; that effective learning in community-based initiatives may not occur by simply adapting models, plans, and frameworks from other contexts, but by addressing key conditions that are necessary for stimulating and mobilising learning process. These are presented in this paper as key findings and described further using theoretical insights.

A coastal strip that extends from Moa to Boma localities in Mkinga District (Figs. 1 and 2) was picked as the main study site on grounds that it would provide opportunities for selected research participants to work on the environment that they are familiar with, rather than being moved to one specific location. Selection of the site was based on the following criteria:

1) It contained over one thousand hectares of mangrove species with varying ecological characteristics (good for experiential learning);

2) Resource users in the study area had been involved in coastal monitoring practices from the early 1990s and were thus well versed with challenges that are associated with adapting and applying the framework of scientific indicators;

3) The researchers had participated in various community based natural resource management in the study area for over two years and in other coastal sites for over ten years, and therefore, potentially had access to useful information for the intended study.

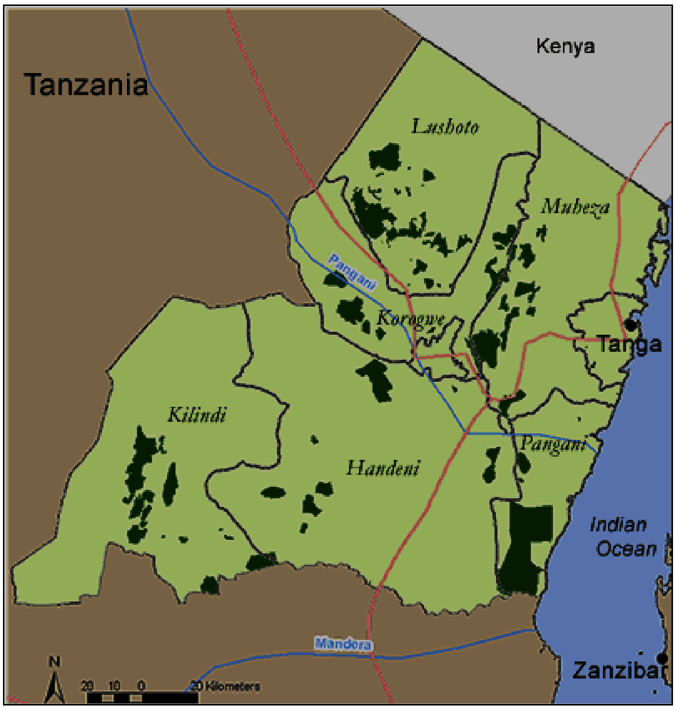

Figure 1: The study area.

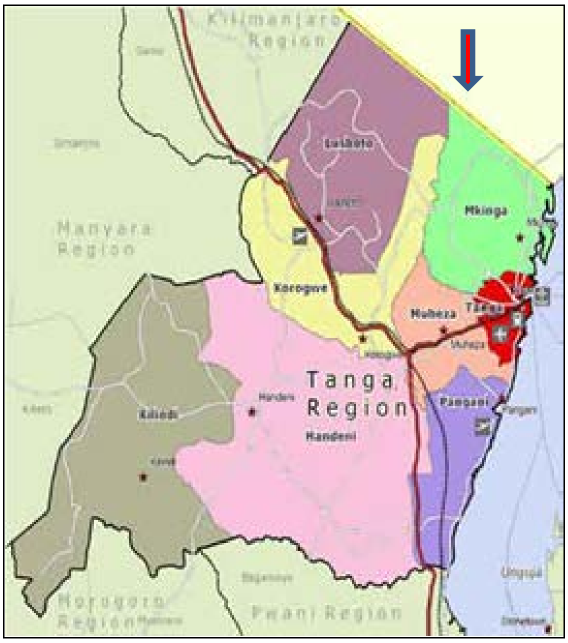

Figure 2: Mkinga District.

MATERIAL AND METHODS

The study was carried out in a mangrove ecosystem restoration context in the eastern coast of Tanzania to “analyse” possible challenges that enable or constrain learning in participatory monitoring of coastal and marine resources. The study also sought to “examine” existing local knowledge as an input for underlaboring (supporting) the existing scientific framework of indicators in response to the challenges raised by research participants (coastal communities) and the advice given by some scientists that “local input” is required when developing participatory monitoring indicators in order to “accurately measure what is locally important” (Fraser et al., 2005).

It pursued a case study strategy to allow the investigator to “retain holistic and meaningful characteristics of real-life events” (Yin, 2003) that occurs in the mangrove ecosystem. Opting for this strategy also implied choosing to have a deeper understanding of phenomena under study (depth) than how wide they are (breadth). Case study research yields either “descriptive” or “explanatory” knowledge (Babbie, 2001, 2007).

The sampling process (in this study) was guided by Verschuren and Doorewaard (1999) who recommend the use of a “strategic” sample to allow in-depth analysis of the phenomenon under study. Selection of research participants was based on their previous involvement in mangrove and fisheries practice in the study area. Using this criterion, the study selected fishers who had participated in fishing activities for at least 15 years, mangrove restorers with at least ten years record and local elders who had witnessed trends, threats, changes and conditions of fisheries and mangrove resources for at least 20 years. Using previous contacts, the researcher formed a team of five experienced mangrove restorers and fishers (three women and two men) to assist in the process of selecting potential research participants in the study area. The team identified eight elders, six fishers and 12 mangrove restorers whom they believed to have sufficient knowledge of the mangrove ecosystem as per specified sampling criteria.

Analysis of challenges that emerge from the scientific framework of indicators which enable or constrain learning in participatory monitoring of mangroves and fisheries

The analysis of challenges that enable or constrain learning in participatory monitoring of coastal and marine resources was done through Experiential Learning Intervention Workshop (ELIW) which allowed mangrove restorers, mangrove-based fishers, local elders and marine scientists to mirror the scientific framework of indicators and specifically analyse challenges that are associated with the adaption and application of the same.

Procedures adapted in the ELIW

The intervention workshop was divided into four main sessions. The first session focused on analysing the level of familiarity and comprehension of attributes that constitute the framework of scientific indicators for monitoring mangrove species and mangrove-based fisheries respectively. The second session focused on identifying and associating scientific monitoring methods and techniques to the attributes or indicators identified during the first session. The third session aimed at examining the capacity of the participants to apply the framework. This session focused on capturing challenges that users of the framework experienced in the process of applying the scientific framework of indicators in the field and recording of the same in special sheets. The first two sessions were introduced to pave the way for the participants to recall various monitoring moments they had gone through, and be able to mirror different challenges that either enabled or constrained them from acquiring knowledge as they took part in the practice. The fourth and last session aimed to analyse the relevance of the indicators to the local context, identify emerging errors, correct observed errors, add local input to improve the framework and produce a user friendly framework (which is contextually relevant and cultural friendly), test the new framework, and provide feedback of the testing exercise. (Tabs. 1-4; Box 1)

Table 1: Level of familiarity and comprehension.

Crt.	Checklist enquiry	Responses given	Total participants	Percentage	Reason(s) For “Yes” or “No”
1.	I understand the components of the framework	2	20	10	My little formal knowledge was enough to help me, I have been part of different training workshops.
2.	I partially understand the components of the framework	5	20	25	I can catch-up with some components.
3.	I don’t understand the components of the framework	13	20	65	Used language is difficult, too many components, I have no formal knowledge, I don’t know how to read and write, I don’t know why I can’t understand them.

Table 2: Applying the framework of scientific indicators for monitoring mangroves.

Crt.	Checklist enquiry	Responses given	Total participants	Percentage	Reason(s) For “Yes” or “No”
1.	I am able to apply the framework	2	20	10	Too difficult to understand, I have no formal knowledge, not able to read and write, I don’t know.
2.	I can partially apply the framework	8	20	40	We don’t use them on regular basis, they are difficult, they are too systematic
3.	I am not able to apply the framework	10	20	50	We did not develop them, they contain mathematics, and they are difficult.

Table 3: Applying the framework of scientific indicators for fisheries.

Crt.	Checklist enquiry	Responses given	Total participants	Percentage	Reason(s) For “Yes” or “No”
1.	I am able to apply the framework	9	20	45	None
2.	I can partially apply the framework	3	20	15	None
3.	I am not able to apply the framework	8	20	40	Some of the components are not clear to us.

Box 1: Challenges about the scientific indicators.

1. “They are good but you need time to understand them.”
2. “They should be revised.”
3. “They are based on books.”
4. “They are for those who went to school.”
5. “They should be simplified.”
6. “They are cost oriented.”
7. “They are difficult to follow.”
8. “They can be improved.”
9. “They are for educated people.”

Table 4: The scientific framework of Indicators applied in the study area since 1990s. (Source: English et al., 1994, 1997; Hill et al., 2005; KICAMP, 2005).

Scientific attributes/indicators for monitoring mangroves	Scientific attributes/indicators for monitoring mangrove-based fisheries
• Community structure and Biomass • Primary Productivity • Leaf litter production • Soil characteristics • Area coverage • Species composition and diversity • Dynamics (change)	• Population size • Population structure • Breeding success • Weight and length of fish by species • Type of gear used to catch the fish • Distance to fishing ground • Type of vessel used and size • Means of vessel propulsion • Number of crew • Incidental catches of endangered species

RESULTS AND DISCUSSION

Challenges raised from the Experiential Learning Intervention Workshop suggest a list of conditions that determine or necessitate the adaption of scientific knowledge in a mangrove monitoring scenario. These include the level of education that the participating group has, level of participation opted for, contextual realities on ground, consideration of the potential input that target communities have, and the level of structures opted for.

Level of education

One of the conditions that require attention is, for example, the capacity of the target communities to adapt and apply scientific methodologies. This appears to be necessary since literacy levels, especially in the developing countries, vary across regions. In one of the studies carried out in the eastern coast of Tanzania by a team of marine scientists, a random sample of 145 individuals was taken for purposes of analysing their socio-economic status, including the level of education. The results indicated that the level of education among target communities was very low. About one-third of the population (31%) had no formal education at all, 26% had reached only up to standard IV, 3% had completed standard VII, and only 2% had received post-primary education. The study also revealed that women had generally "lower literacy and educational levels" than men (Wagner et al., 2001).

In another coastal monitoring context, an assessment was carried out in the same area by one of the capacity building programmes through a two-day participatory workshop to establish the capacity of coastal communities and local government staff to understand and apply the adapted monitoring plan. It was observed that the latter were more conversant with the indicators and methodologies used in the plan than the former (KICAMP, 2004). The difference was scribed to the varying levels of formal education between the two. The level of "practical experience" amongst the two groups could not be taken as a confounding factor since both participants were exposed to the plan at the same time and had no prior knowledge of the same. This suggests that there is a close link between literacy and numeracy skills and the capacity to adapt and apply scientific indicators and methodologies. In other words, understanding is a prerequisite for taking part effectively in the practice.

Reed et al. (2010) argue that for learning to occur in a community of practice (as it is the case with the participatory coastal monitoring initiatives), involved individuals must demonstrate a change in “understanding”, and be able to share their knowledge with other participants to the level that will attract more involvement and wider participation of other community members in the same practice.

Level of participation

The second condition that comes out so strongly in the community-based monitoring is deciding the level of participation that is required for community involvement. This involves taking part in choosing educational tools that are suitable in the learners’ context. It depicts a doctor-patient scenario where involvement and willingness of the patient in the prescription process is necessary. Some facilitators tend to assume that target communities (learners) may only be involved at particular levels of the initiatives, whereas others do not involve them at all (Songorwa, 1999). Campbell and Vainio-Mattila (2003) insist that target communities should influence the conception, design, and implementation of the introduced initiatives. Influencing the conception and the design implies taking part in deciding the structure, approach, materials that suit their need and situations that address the actual reality in a coastal and marine setting.

There are two options that are normally preferred by facilitators when laying down strategies for participatory learning initiatives at local level. The first option is to use adapted tools such as models, framework of indicators, plans, and any other interventional methods or techniques (KICAMP, 2005). This approach is common and mostly favoured as discussed earlier in the introduction part. The second option sets opportunities for target communities to create their own monitoring tools depending on conditions that prevail in their context and thereafter apply them. The latter option seems to carry on board the real meaning of participation or “participatory approaches” and is probably least favoured. Choosing either of the two needs to be decided and agreed by involved individuals and social groups in every-community based scenario. Comprehension of the said tools emerge to be an important factor since as discussed earlier, learning can hardly occur without understanding (Reed et al., 2010). Campbell and Vainio-Mattila (2003) argue that communities are not “passive bystanders” in the on-going or introduced initiatives but have to be actively engaged in the negotiations that determine what those initiatives will look like.

Contextual realities on ground

It is observed that long-term changes in the initiatives that local communities are involved in may only be realised or experienced if an emphasis will be laid on “situated knowledge”. Campbell and Vainio-Mattila (2003) defines this as “a process whereby access to the information, and control over knowledge use shifts from experts and scientists to the people whose lives are being affected”.

Situating knowledge in a particular learning context requires consideration of various elements. Indicator development for the monitoring of mangroves as a practice that stimulates learning should thus be rooted in contextual realities. Arguing in favour of prioritizing contextual information Glahn et al., (2007) provide the following description: “Actors depend on indicators in order to organise, orientate and navigate through complex environment by utilising contextual information. Contextual information on the learning processes has been proven as important to support the learning processes. It stimulates the learners’ engagement in and commitment to collaborating processes; it helps to raise awareness of, and stimulates reflection about acquired competences; it supports thoughtful behaviour in navigation and learning paths.”

Glahn et al. (2007) not only insist on developing indicators that are relevant to the context within which they will be applied, but also bring into view the fact that the processes which lead to development indicators are closely linked to learning. In other words, it implies that involving local actors such as coastal communities in such process or practice creates opportunities for them to learn from each other, and therefore understand better both the context and the indicators they have developed. This may also suggest that imposing or causing actors to adapt indicators from other contexts deprives them of opportunities for learning and compels them to apply tools that are not their own creation.

According to ITAD (1997), the measures that the indicators suggest must be contextually appropriate, clear and acceptable to target communities to avoid misrepresentation of information, over-reporting or underreporting of events. The indicators should also be cost-effective, relevant to the context, easy to apply or use. Scheltinga et al. (2004) also emphasize the need to avoid complexity by also avoiding technical abstractions and embarking on simple processes that can be easily be measured, analysed, and interpreted by involved communities.

Rydin et al. (2003) noted a wave of change in indicator development, from a “technical” process which involve experts (at global level) to a participatory process which focuses on understanding the local context within which the indicators are being developed as a process which focuses on the relationship between lay people and experts. They argue that if indicator development is no longer a technical issue, then it should not be left to experts, but rather to people who are directly affected by the situation. Indicator development should thus be centred on the learner’s situation or context, and not on “static” approaches which according to Glahn et al. (2007) follow a fixed set of rules in the process of collecting aggregating and indicating information to learners.

As indicated earlier, comprehension is a key element in the learning process without which learning of whatever kind can hardly be attained or attracts a wider level (Reed et al., 2010). Reed et al. (2006) identified two key criteria for indicators: ability of users (learners) to apply them and their relevance at local level as discussed earlier. For users of the indicators to meet the first criterion (ability to apply them), they must first comprehend them. The level of comprehension amongst them will depend on the level reification and abstraction that underpin their involvement in the initiatives they are undertaking.

If for example, the development of the said indicators pursued a formal natural science route, common people who have never had access to participate in “formal learning” can hardly understand them. But if the abstractions favour the local context and the learners or users are part of the process that leads to development of such indicators, they are likely not only to understand the indicators but interact and share new knowledge with other local actors.

It is also wise to learn from target communities whether or not there are cultural aspects that need to be addressed or considered in the monitoring plans prior to adapting any frameworks or tools. This is an important aspect since culture in most societies and is closely linked or related to moral aspects (Medin and Atran, 2008). When it is regarded as a moral duty, it may create a binding situation which compels a defined community to abide by what is believed to be “morally” acceptable. In this manner, culture may either act as an enabler or constraining factor. For example, if mangrove forest sites are regarded or designated by a particular local community as being sacred, visiting such areas may be restricted to specified individuals and opportunities for other member of the community including the facilitators to undertake conservation or management activities, may be limited.

Development of monitoring indicators should thus consider what may be acceptable in a particular culture and what may not. This can be reached by consulting target social groups and encouraging processes that will allow them to take part at all levels of the monitoring plan, as well as through the implementation process.

The level of structures opted for

The kind of structures preferred by facilitators may well affect the process of developing community-based monitoring programmes and ultimately enable or constrain the learning process. Reed et al. (2006) present two paradigms that determine the development of indicators in the local context as being the top-down and bottom-up. The process that leads to development of indicators under the top-down paradigm tend to exclude contextual aspects and do not encourage consultation of local communities as does the bottom-up approach. Indicators that result from the latter “provide a more contextualised understanding of local issues” and guarantee sustainability, ownership and accountability of participating learners. Fraser et al. (2005) remind development experts (facilitators) to ensure that the process of choosing indicators should consider their relevance to local situations.

Consideration of local input

There is evidence that local communities that are involved in participatory monitoring of coastal and marine resources are hardly asked to share experiences and situated knowledge for purposes of informing the conception and implementation of planned initiatives. Campbell and Vainio-Mattila (2003) present two cases where marine scientists “continued to rely heavily on western scientific criteria” in determining conservation practices.

Fraser et al. (2005) insist on the fact that “local input” is required when developing community based monitoring indicators in order to “accurately measure what is locally important”. Commenting on the need to consider local knowledge in the monitoring process Berkes (2012) states: “As people with detailed understanding of the environment and accumulation of observations over generations, indigenous groups have a special place in community-based monitoring. As well, it is becoming clear that many indigenous groups have developed their own traditional monitoring based on their own ways of knowing. Most traditional monitoring methods used by indigenous people are rapid, low-cost, and easily comprehensible by harvesters themselves as they hunt, fish, and gather the forest products”.

Berkes (2008) observes that insights of indigenous wisdom offer great potential for broadening epistemological access, given the difficulties and limitations of accessing and using scientific knowledge in addressing complex ecological challenges. Such difficulties emerge when scientific institutions favour the language of description and methodologies that are too hard for non-specialists at community level to follow, leading to lack of epistemological access between scientific institutions and local communities (Reed et al., 2006).

Reid et al. (2006) advise that ways have to be explored in which scientific (western) and traditional knowledge can be used together, and conditions necessary for such integration need to be better understood.

Developing of an alternative framework of indicators as under labourer for the existing scientific framework

To examine the existing local knowledge as an input for addressing various challenges that are associated with the adaption and application of scientific indicators, open-ended questionnaires were administered to selected mangrove restorers, fishers and local elders to guide in-depth individual interviews that would yield traditional ecological knowledge on the trends, changes, threats and condition of mangroves and fisheries that use them as key habitats. Such forms of inquiry are applied when researchers seek to capture in-depth data in specific cases (Boyce and Neale, 2006). Interviews with individual fishers were carried out near fish-landing sites to allow them to choose at least three kinds of mangrove-based fisheries as objects for abstraction and representation. Interviews with individual mangrove restorers were held near or in the mangrove sites depending on the tide level and ability of participants to cross muddy areas to desired positions (where they could clearly see or touch mangrove species while they continued to describe them). Interviews with the local elders were held in their home compounds or near mangrove sites depending on age, physical well-being, and preferences.

The interview processes for each of the participants' groups (fishers, mangrove restorers, and elders) were culminated in three Focus Group Discussions (FGDs). Berkes (2008) argues that individual knowledge and experience tend to be distinct, but it is enriched by the knowledge of the group and is "shaped by the ways of knowing of the group". It is on this ground that this study opted for FGDs as a second medium of enquiry or a culminating-point for individual abstractions, conceptualisations, and representations of traditional ecological knowledge (TEK). FGD inquiry was also considered useful in this context since it allows researchers to probe, places research participants in natural or real situations, can be easily understood, and has high "face validity" (Krueger, 2000). FGD also provides speedy results, and it follows socially oriented procedures. Moreover, the method allows the researcher to gain information on "how people think, perceive, give ideas, and share experiences" (Wilkinson, 2000).

Analysis of Traditional Ecological Knowledge (TEK)

Emerging data from individual in-depth interviews, focus groups and experiential learning cycles were captured and coded into specific themes/sub-themes. This process was followed by assigning participants' representations to emerging themes or sub-themes and analysing their ontological or epistemology nature based on local experiences. The focus was to examine the potential contribution of traditional ecological knowledge to the development of monitoring indicators that incorporate contextual, social and cultural realities.

Key themes emerging from the processes of coding included: (1) the physical **condition** of mangroves and fisheries that use them as habitat, (2) **threats** that face the mangrove ecosystem in general, (3) **changes** that occur in the mangrove ecosystem, and (4) **trends** of mangrove and fisheries species in the mangrove ecosystem. Obtained data were further discussed by research participants, assigned to the four (4) key themes and used to develop a draft framework of Traditional Ecological Indicators (TEK) which had the potential of being added or integrated with the existing scientific framework for the purposes of enhancing learning. This was carried out through series of four Focus Groups held in the study area between January and March 2012. The successful development of the draft TEK framework uncovers the curiosity of mangrove restorers and fishers to respond to the challenges they had experienced earlier while applying the scientific framework of indicators and associated methodologies (Tabs. 1 and 2; Box 1), which affect participatory monitoring practices and limit learning in the eastern coast of Tanzania.

Attributes developed by coastal communities for underlabouring (supporting) scientific mangrove indicators

- Drying or withering of mangrove species;
- Presence of stumps;
- Soil type;
- Presence of invasive species;

- Presence of charcoal kilns;
- Presence of saltpans;
- Physical outlook of plants (leaves, roots, stem, branches, epical bud, shoots);
- Dense canopy;
- Accumulation of solid waste in the mangrove;
- Presence of invertebrates and vertebrates.

Description of the attributes/indicators as perceived by coastal communities

a) Drying or withering of mangrove species

Drying or withering of mangroves is associated with human induced threats, disease or natural disasters occurring in the coastal areas such as tsunami, invasive species, and deprived nutrient levels. Human induced factors can be mangrove cutting for domestic and commercial use, root digging in search of baits (mainly earthworms) and accumulation of solid waste around young plants. Monitoring techniques that can lead to drawing conclusion that the plant has withered/dried includes; physical observation and inspection of leaves, branches, and the stem. A mangrove plant that is under this kind of threat will indicate a condition of dried/drying leaves, falling of leaves on ground and possibly drying branches.

b) Presence of mangrove stumps

The presence of mangrove stumps in the mangrove forest indicates that there has been mangrove clearance in the area. Stumps can indicate the extent of damage and suggest the time when such threat occurred. This is possible since old stumps are dry and their colour is different from the new stumps. The level or extent of damage will be established by counting down the number of stumps and by making strides (counting human steps). Identification of the affected mangrove species is also possible and can be done by observing neighbouring species. If nearby species are Mchu/Mchwi (*Avicennia marina*), then the damaged species will be the same, since species of the same kind or type tend to grow together.

c) Soil type

Soft-muddy soils allow plant-roots to penetrate deeper in the ground and absorb nutrients. Plants that grow in this area are likely to be healthy. Their leaves remain green and their heights higher than those in rocky areas. On the other hand, rocky areas suggest that mangrove roots cannot easily penetrate the ground. Such species will appear short, with roots coined on top of the ground and colour of their leaves will remain between green and yellow. Rich soil will also show black colour while rocky and poor soil will depict a yellowish or reddish colour. The indicator for the health status will be the type of soil, height of plants, soil colour and colour of leaves. Involved techniques will involve examining whether the soil is soft-muddy or rocky and indicating it in the report form and carefully identifying the colour of leaves and stating it, and thereafter use agreed indigenous measures (pima) in establishing the plant height.

d) Presence of invasive species

Presence of invasive species suggests that mangrove plants are subject to competition for nutrients with species that do not belong to their colony (intruders). These may encroach on the mangrove from nearby ecosystems, or may be caused and accelerated by other factors such as human activities or natural causes. To determine whether there are invasive species in the mangroves, monitoring teams can do physical observation around different mangrove species, and inspecting specific parts such as plant canopy, the ground and any other possible areas. Species that are not of mangrove origin can be seen or identified by naked eyes. Other identification techniques include smelling, and comparison (comparing their leaves with mangrove species).

e) Presence of charcoal kilns

The presence of charcoal kilns in the mangrove forest suggests there has been clearing of plants in the area and it is therefore a good indicator that destruction of mangrove species has occurred. The extent of damage will depend on the number of charcoal kilns seen in the area and also the number of stumps that may be associated with such a clearance. The most affected species can also be identified by observing their proximity to their “mother colonies”.

f) Presence of saltpans

Like charcoal kilns, the presences of saltpans indicate that there has been a significant clearance of mangrove species in the area. Saltpans require enough land-space for salt making; and mangrove areas prove to be suitable for this kind of economic activity since they allow lodging of saline water from the ocean; which is exposed to evaporate under controlled environment, leaving behind salt as an output or product. Establishing the extent of damage can be done by counting the number of saltpans and striding the size of each saltpan. Counting stumps is not possible in this case since mangrove clearance for saltpans is normally accompanied by uprooting and levelling of the area. The most affected species can also be observed and recorded.

g) Look of the plant species (leaves, roots, stem, branches, epical bud, and shoots)

Physical look of the plant is among the key indicators of good or bad plant health among indigenous coastal communities. Plant leaves, roots, shoots, stem, branch, epical bud and length are themselves independent indicators which can show the status of a plant.

Leaves. Mangrove leaves range from green to evergreen colour depending on the species type. Change in colour from evergreen to yellow, pale, or red, suggests that either there is a change in season or a threat from both natural and human induced factors. Monitoring teams may closely make an inspection to determine the cause for change in colour and establish whether it is a result of poor soil, human threats or other causes.

Roots. Plants with roots that are fully covered by the soil are considered to be stable and safe from human induced activities and wild animals. Exposed roots indicate a short life-cycle of a plant since roots hold and keep it in contact with the nutrients from the soil. Fishers tend to dig out earth worms from mangrove roots as baits that might attract particular fish species. Wild animals also dig out various worms for food. Carrying out a careful inspection beneath mangrove species can lead to establishing whether the roots are fully covered or partially covered, fully exposed or partially exposed. The status of identified species will be established based on the above factors.

Stem. Plant stem indicates its width and level of nourishment. A good looking stem suggests that the entire plant is likely to be healthy. Peeling of tree-bark around the stem may lead to mortality of mangrove species. The stem is regarded as a store for the plant food. Its damage or destruction may lead to starvation of the plant and ultimately death. Physical inspection of the stem (damaged or undamaged) and its size (width) may help in establishing the plant’s health status. A clear observation of the plant-bark to identify possible damages and likely cause will also help. Measuring of stem-width can be done by using a tape measure or traditional measures (pima).

Branches. Straight branches with aligned bark-thickness show continuous growth. Past records in Mkinga show that mangrove branches are selectively cleared for boat making and construction of residential apartments. In such a process, young branches are damaged and dwarfed. Branch straightness and the presence or absence of remarkable damage in mangrove branches in selected mangrove plots will help in establishing their health status.

Epical bud. Removal of the epical bud suggests a limitation of plant’s growth upward, hence limited length. The presence or absence of the epical bud therefore will determine whether a particular mangrove plant is growing upward or not. Inspecting plant canopy or top will help the monitoring team to establish whether the epical bud is present or absent.

Plant shoots. The presence of shoots around the plant suggests a promising future of the forest-growth or recovery from threats. The absence may suggest an ageing forest or seasonal changes. Plant shoots in specified plots will be traced and counted. A second inspection will be done to establish whether they are sparsely distributed or concentrated in particular plots, followed by a decision whether they indicate a good or bad mangrove health.

h) Dense canopy

A good aligned canopy with a distinguished green colour suggests that the forest is in good health. Poorly spaced mangrove species depict a spaced canopy that is only good in some parts of the forest and poor in other parts. Appearance of the forest canopy from at least a distance of 300 m, the number of bare spaces in the identified plots and the general outlook of the forest colour will help in establishing the health status of the mangrove forest. Monitoring techniques will thus involve standing within a range of at least 300 m away to observe the appearance of the canopy and counting bare spaces in the specified plots and ranking the canopy as either good or bad.

i) Accumulation of solid waste in the mangrove

Accumulation of solid waste in the mangroves impairs growth of plant-seedlings and shoots. Discarded fishing nets, clothes, plastic materials, ropes, broken boats/fishing vessels and other forms of waste that can trap seedlings and shoots are harmful to the mangroves and also to other vertebrates and invertebrates, such as juvenile fish, that use mangroves as a habitat. Observation of the presence or absence of solid waste in the mangroves will therefore help to establish whether such a threat exists or not. It may also be possible to locate the source of waste in order to address the threat.

j) Presence of invertebrates and vertebrates

The crawling organisms presence in the soil/ground that harbours mangrove species may suggest threats to seedlings and plant shoots. Other organisms like worms will generally indicate good aeration of the soil. A notable indicator in this case will be the presence or absence of insects or any other marine organisms that are considered having positive or negative relationship with the plant. A careful observation and identification of the encroaching organisms near mangrove species and particularly in the shoots, seedlings, and roots will be required.

Indicators developed for underlabouring scientific fisheries indicators

- Gills colour;
- Colour of eyes (pale/brownish are bad);
- Skin condition (soft/hard) bouncing back or not;
- Presence of wounds/scars/bruises;
- Weight in relation to fish size;
- Condition of the air bladder (good or bad);
- Presence or absence of body fats;
- Fish shape (physical appearance);
- Catch frequency of various species (most fished fisheries/least fished);
- Frequency of blasts in case of dynamite fishing;
- Number of fishers involved in mangrove fishing in the area;
- Body parts (missing or damaged).

Description of the attributes/indicators

1) Gills colour

The normal gill colour for a fish that has just been fished-out is supposed to be reddish. If the gills display a pale or whitish colour then the monitoring team may consider that particular species as unhealthy. Possible assumptions for the bad health status may include the possibility that such a fish was already weak before being fished out. Traditional methods or techniques for detecting the status of gills include physical observation of the gills in selected fish-landing sites and recording observations in a monitoring sheet.

2) Eye colour

Colour of the fish-eye may indicate whether particular species is healthy or not. Pale and brownish colours suggest poor health. Besides those two “bad colours”, different fish may have different appearance of eyes depending on their types. Key methods for accessing information that capture such an indicator is to observe caught fish in selected fish-landing sites and involve experienced fishers who can easily distinguish eye colours of different fish species.

3) Skin condition

Skin may also indicate the health status of a fish. A healthy fish that has just been fished out, when pressed hard, its skin bounces back to its original form. Unhealthy fish when pressed with the thumb does not bounce back, but rather attaches itself to the bones/flesh and leaves the thumb’s mark in the pressed area. Identifying healthy fish through this indicator can be done near fishing-land sites. Other collected information that may go along with the above indicator may include recording of the species type and whether it is a juvenile or an adult fish. This may help to explain the category of affected species and guide management plans.

4) Presence of wound/scars/bruises

The presence of wounds/scars/and bruises in a fish that is displayed in the fish market or landsite suggests that a particular fish is not healthy. Wounds may suggest that the concerned fish species might have sustained injuries from hooking, dynamite blasts, or any other causes, including escape from natural predators, and that its health status must thus have been affected. Confirmation of the fish health status can therefore, be done by examining whether there is any piece of hook hanging around the throat and in the stomach, and the presence of wounds, scars and bruises. This physical inspection or observation has to be carried out in a nearby or designated fish-landing site. Records of availability of scars and possible cause of injuries will be recorded in the monitoring sheet or form.

5) Weight in relation to fish size

Fishers believe that fish weight is proportional to its size. Any mismatch may suggest that there is a health problem. Fishes with certain length are expected to weigh according to fisher’s expectations. This requirement varies among species. Mangrove-based fish species such as mudskipper have average length and weight than can easily be estimated by local fishers. Physical observation of caught fish may be verified by weighing them out and taking metre-measurements. After that, fishers may link fish-weight relationship to their health status.

6) Condition of the air bladder

A damaged fish airbladder can be an indicator for fishing with dynamite. When the bladder is intact, it indicates that fish has been fished out using common fishing gears. Confirmation of using fishing gear may be done through physical inspection of fishes in a landing site. Hooked fishes will show scars. A damaged air bladder is mainly used in establishing whether dynamite fishing was used or not. Information as to whether the fish airbladder was intact or damaged can be recorded in special forms/monitoring sheets.

7) Presence or absence of body fats

The presence of fats in a fish suggests that it is in good health. On the other hand, fish that lack fats in their body are described as being skinny and unhealthy and even not suitable for human consumption. To detect body fats, fishers may look around the abdomen and examine the fish body alignment. Breeding fisheries are regarded as having such indicators and are thus good in health. In instances where the monitoring team may not be able to directly use local experiences to establish whether a particular fish has body fats or not, the abdominal part may be parted (using agreed devices such as a sharp knife) to see what is inside and establish the premise. Records of the number of fattened-fish against those which are not can be taken by the team.

8) Fish shape

Physical appearance of caught fish or their shapes may indicate their health. Thin, small and dormant fish species are generally regarded as being unhealthy. Big and huge species are linked to good health status. If, for instance, mullet species are by average huge, any caught mullet species that may appear long but thin, unlike the majority of other species will be considered as having poor health. Collecting information for the above particular indicator may involve physical observation of fish in designated land sites or markets. Records that are associated with size may be recorded using tape measures, and weighing measurements.

9) Catch frequency of various species (most/least fished fish species)

Catch frequency may be used as an indicator for species abundance or decline. Those that are caught on regular basis may be regarded as being abundant and those that are rarely fished may be regarded as “endangered”, and therefore, inform management or conservation decisions. Collecting information or data that indicates catch frequency per species can be done at fish landing sites or markets, using special forms or sheets.

10) Frequency of blasts

Blasts frequency in ocean indicates that “dynamite fishing” is likely to be taking place. This information can be collected by counting blasts heard per day or week. Some members of the monitoring team may be assigned the role of recording frequencies of dynamite blasts and report cumulative blasts during periodical monitoring of fisheries resources.

11) Number of fishers involved in mangrove fishing in the area

The number of fishers involved in mangrove-based fishing may indicate whether there is fishing pressure in the mangrove or not. A small number may suggest that fish-growth is assured, as more juvenile fish in the mangroves (as nursery grounds) are allowed to grow to maturity level and also the possibility of having breeding species which spend some time in the mangroves in spawning seasons to survive longer. More fishers, on the contrary, may suggest a future decline in fish catch and possibly “extinction” of mangrove fisheries. Information or data about this particular indicator can be accessed by making inquiries among fishers about the number of fishers who are specialized in mangrove fishing and keep on updating the list periodically.

12) Body parts (missing or damaged)

Some fish may have missing or damaged body parts such as tails, gills, fins, eyes, or cut mouth. These may suggest that involved fisheries are physical impaired and are therefore, not considered as being healthy. Possible causes for this condition include, dynamite fishing and the use of unfriendly fishing gears. Fisheries with missing or damaged parts can be directly observed from nearby fish-landing sites and recorded in special forms. Records should indicate the affected part, species type, and age (juvenile or adult).

Table 5: Integrated monitoring framework for mangrove (TEK and scientific indicators).

Crt.	Attribute	Indicator	Nature of the indicator	Monitoring technique/method
1.	Soil characteristics	Soil type and colour: Muddy-black soils indicate high nutrients; rocky reddish or yellowish indicate poor soil.	TEK/Scientific	Physical observation of soil layers and colours and recording observed details.
2.	Species composition and diversity	Area coverage	Scientific/TEK	Dividing mangroves into specific plots, taking strides/metre measurements of plot size, counting individual plants per plot, observing and recording dominant species per plot and recording total young and mature species.
3.	Dynamics (change)	Physical outlook of plant species: leaves, roots, stem, branches, epical bud and shoots.	TEK	Observing leaf-colour whether is ever-green (good), pale/red (poor) and yellow (very poor), indicating the cause of change (e.g. poor soil, human threats, or natural). Observing whether the roots are fully covered by the soil, exposed or partially exposed; check the stem (is its bark peeled off or not); any threats to the branches, and shoots? Epical-bud removed or not?
4.	Presence of mangrove stumps in area	Number of stumps in the mangrove area	TEK	Counting the number of stumps, taking strides or meter measurements to establish the size of damaged area.
5.	Drying or withering mangrove species	Number and type of affected mangrove species	TEK	Physical observation of leaves, branches, and stem per plot and recording observed condition.
6.	Presence of invasive species	Type of invasive species, size of the area covered, and number of affected mangrove trees	TEK	Inspecting specific parts such as the tree canopy, the ground recording observed details. Area covered by invasive species can be measured by metre or strides depending on the affected part.

Table 5 (continuing): Integrated monitoring framework for mangrove (TEK and scientific indicators).

Crt.	Attribute	Indicator	Nature of the indicator	Monitoring technique/method
7.	Presence of charcoal kilns	Number of charcoal kilns in the mangrove area	TEK	Physical observation and recording of details.
8.	Presence of saltpans	Number of saltpans in the mangrove area, size of the damaged area.	TEK	Physical observation of the affected area and taking strides or metre measurements to establish the size of the damaged area.
9.	Dense canopy	Canopy appearance (thick or poorly distributed), and number of open spaces/gaps in the mangrove per plot.	TEK	Observing the appearance of mangroves from at least a distance of 300 metres, and counting the number of open spaces/gaps per plot.
10.	Solid waste	Accumulation of solid waste in the mangroves	TEK	Observing the presence or absence of solid waste in the mangrove and classifying the kind of waste trapped in the mangroves (e.g. plastic, broken boats, discarded fishing nets, clothes, ropes, etc.) and indicating affected mangrove species
11.	Presence of invertebrates and vertebrates	The kind of vertebrates or invertebrates present under the mangrove tree	TEK	A careful observation and identification of crawling organisms around the plant, particularly in the roots, shoots and seedlings.

Source: Developed from field data/ELIW 2012.

Table 6: Integrated monitoring framework for mangrove-based fisheries (TEK and scientific indicators).

Crt.	Attribute	Indicator	Nature of the indicator	Monitoring technique/method
1.	Weight and length of fish by species	Fish length and weight	Scientific/TEK	Weighing caught fish per species, using a metre to measure their length, and comparing recorded weight of selected species by size to observe possible differences
2.	Used fishing gear	Type of gear used to catch fish, (unsustainable gears suggest threats to fisheries or high mortality rate while sustainable gears indicate assured future birth-rates).	Scientific/TEK	Asking fishers to help in identifying used fishing gears and recording them.
3.	Distance to fishing ground	Distance in metres or kilometres from the take-off point to the fishing ground	Scientific/TEK	Use of “estimation method” to establish possible distance or metered boats when such equipment are available
4.	Mangrove fishers in the area	The number of fishers involved in mangrove fishing in the area	TEK	Physical observation (counting) and seeking confirmation from fishers in the area.
5.	Incidental catches of endangered species	Number and type of endangered species caught	Scientific/TEK	Interviewing fishers in fish-landing sites and recording given information or data.
6.	Gills cover	Colour of gills (red colour suggest good health while pale and whitish indicate not good or bad health)	TEK	Physical observation of the gills at a fish-landing site and recording the observed colour.
7.	Eye colour	Colour of fish eyes (pale and brownish suggest poor health)	TEK	Observing caught fish in fish-landing sites, and involves experienced fishers in the colour identification.

Table 6 (continuing): Integrated monitoring framework for mangrove-based fisheries (TEK and scientific indicators).

Crt.	Attribute	Indicator	Nature of the indicator	Monitoring technique/method
8.	Skin condition	Ability of the “skin” to bounce back when pressed/ thumbed. Skin of a healthy fish bounces back when pressed as opposed to unhealthy one.	TEK	Pressing caught fish with thumb at a fish-landing site to observe whether the skin bounces back or not, and recording the observation. Species type/age (juvenile/mature) may also be recorded to indicate the affected species.
9.	Wounds/scars/ bruises	Presence of wounds, scars or bruises indicate a health threat.	TEK	Inspection or observation of wounds, scars, or bruises in a nearby fish-landing site, and any other threats such as hanging hooks.
10.	Condition of fish air-bladder	A damaged fish-airbladder indicates the use of dynamite in fishing	TEK	Physical inspection of the fish-airbladder in a nearby fish-landing site with the assistance of experienced fishers. Recording the affected species by type and age (juvenile/mature)
11.	Fish body-fats	Presence of fats in a fish suggests a good health while those without fats are regarded as skinny and unhealthy.	TEK	Using allowed device (e.g. a sharp knife) to inspect the abdominal part and getting assistance from experienced fishers.
12.	Fish shape	Physical appearance of a caught fish may indicate their health status. Thin, small, and dormant fish are linked to poor health while huge fish are associated with good health.	TEK	Physical observation of caught fish in designated fish-landing-site or markets to establish weather caught fish are regarded as having good shape or not.
13.	Catch frequency	Catch frequency indicate abundance or decline of fish catch.	TEK	Record taking of most frequent caught species to predict a possible future decline and records of rarely caught species for conservations decisions.

Table 6 (continuing): Integrated monitoring framework for mangrove-based fisheries (TEK and Scientific indicators).

Crt.	Attribute	Indicator	Nature of the indicator	Monitoring technique/method
14.	Frequency of blasts	Number of dynamite blasts heard per week	TEK	Interviewing communities and recording the number.
15.	Fish-body parts	Missing or damaged body parts such as eyes, tails and fins.	TEK	Physical observation of caught fishes in the fish-landing-site and recording of missing parts, specie type and species age (juvenile/adult)

Source: Developed from field data/ELIW 2012.

Testing of the new (integrated) framework of indicators and feedback from groups.

This was the last activity in the ELIW cycle, which allowed the participants to form three random groups and choose leaders who would preside over the monitoring exercise at the group level. Each group also appointed a recorder to record observed changes, condition, trends, and threats in the site of their choice. For feedback of the testing exercise, and individual observations see box 2.

Box 2: Feedback made after testing the integrated framework of indicators.

We tested all indicators and recorded the required information as seen in this sheet. We did not face any difficulties. Every member participated effectively in the exercise (leader group A). We went to the eastern part of the forest and read through the indicators before testing. The indicators are easier than those in the old framework. We recorded the required information in the sheet (leader group B).

We successfully tested the indicators although there were some signs of the high tides. We did not finish all the indicators but what we did was successful, there was no problem (leader group C). As we were testing the new indicators, we found them to be simple and easier than the old ones. They are straight forward and can be done in a very short time. All group members played a role in the testing (member group B).

Those indicators were for the educated people. You could count two or three individuals who could at least use them. But these ones are simple because you just observe and record what you have observed (member group C).

CONCLUSIONS

Scientific knowledge emerges from basic and applied research carried out in different contexts and sometimes depicting a specified level of generalization or replication. Though this knowledge can be adapted and applied in various contexts, there is a need to consider factors such as contextual realities on ground, the level of education that the beneficiaries have, level of participation that is required in particular initiatives such as monitoring of the mangrove ecosystem, the potential input that target communities have, and the level of structures that govern participatory monitoring practices in different contexts.

Well-coordinated participatory monitoring practices will not leave target communities as “bystanders” but as active participants at all levels including the decision to adapt, develop or integrate scientific and local knowledge as key ingredients of the monitoring framework. A well-coordinated mangrove monitoring initiative will thus encourage knowledge shift from experts and scientists to the people whose lives are being affected, and at the same time lead to epistemological access, where the former and the latter can share and learn from one another.

While this paper agrees that certain monitoring standards need to be met to allow scientists to share knowledge from different parts of the world, it also place emphasis on the need to secure local actor’s willingness to choose the form of knowledge that will attract their active participation, accountability, comprehension, and application of preferred knowledge. This may greatly reduce or minimize the challenges that this paper brings into view and attract a wider social learning in the coastal environment.

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